Infection strategies of fish ectoparasites

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FISH ECTOPARASITES

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by

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ABSTRACT

Certain factors controlling the parasitic phase of some non-specific fish ectoparasites were investigated. Studies were made of the infection patterns exhibited by the branchiuran Argulus foliaceus (L.), the fish leeches Piscicola geometra (L.) and Hemiclepsis marginata (Mull.) and glochidia larvae of Anodonta cygnea (L.) on freshwater fish.

Aspects of the parasites' life history, survival, development, seasonal cycles in incidence, dispersion patterns, immigration and site selection were investigated by field and laboratory studies.

In adult A.foliaceus and P.geometra, the initial attacking phase onto the fish host does not appear to be influenced by density-dependent factors. In larval A.foliaceus, infection is also unaffected by parasite density, but in adults, it is controlled at high parasite numbers when the carrying capacity of the fish is reached. The infection patterns of A.foliaceus appear to reflect the heterogeneity of both host and parasite populations, particularly with respect to variations in host susceptibility and to parasite searching activity.

In adult P.geometra, the parasitic phase is influenced by density-dependent factors with increasing intraspecific competition at high densities.

The population processes controlling these parasites are compared to other types of host/parasite systems.

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1. INTRODUCTION

Foraging Strategies in Parasites and Predators

Recent studies on predation and insect parasitism have largely been concerned with the way in which organisms are "optimally adapted" in their foraging or searching activities. The implication that predators or insect parasites are "efficient" in their foraging has been discussed in several reviews on feeding strategies (Schoener, 1971; Emlen, 1973; Pianka, 1974; Charnov, 1976a; Cook and Hubbard, 1977). The approaches of various authors relate to the way in which predators directly or indirectly maximize the rate at which prey is obtained, or in the case of insect parasitoids, maximize the number of hosts parasitised.

Schoener (1971) considered that the "primary task of a theory of feeding strategies is to specify for a given animal that complex of behaviour and morphology best suited to gather food energy in a particular environment". One concept inherent in the analysis of the foraging process is that an optimal diet will be selected by the predator. Theoretical models of dietary preference (MacArthur and Levins, 1964; Emlen, 1966, 1968; MacArthur and Pianka, 1966; Levins and MacArthur, 1969; Schoener, 1969a, 1969b, 1971, 1974; Tullock, 1970; Rapport, 1971; MacArthur, 1972; Marten, 1973; Cody, 1974; Katz, 1974; Pulliam, 1974; Werner and Hall, 1974; Emlen and Emlen, 1975; Charnov, 1976a, 1976b; Estabrook and Dunham, 1976) adapt different approaches, but all essentially relate to foraging efficiency and to the utilization of food items by predators. In this sense, an optimal diet is considered to be one which maximizes the net energy obtained per unit feeding time (Emlen, 1966, 1968; Schoener, 1969a,

1969b, 1971; Rapport, 1971; Pulliam, 1974). In discussing optimal diets, MacArthur and Pianka (1966) and Emlen (1966, 1968) consider that the optimum strategy for a predator is one of increased selectivity with increasing prey abundance. One of the problems facing polyphagous predators concerns which prey are taken when a choice of species is available. While a predator may simply take prey according to the relative abundance of the various prey species, more complicated behaviour may arise. One process, namely that of "switching" was discussed by Murdoch (1969) for invertebrate predators. In this situation, if a particular prey species is heavily preyed upon, as that particular prey species declines in numbers, the predator will "switch" to another prey species which may have become more abundant. "Switching" may occur because one prey species becomes more difficult or easier to find in the presence of the other (possibly due to interactions between the two prey species) (Lawton, Beddington and Bonser, 1974). The predator response of "switching" has been discussed by a number of workers (Murdoch, 1969; Rapport and Turner, 1970; Tullock, 1970; Rapport, 1971; Murdoch and Marks, 1973; Harris, 1974; Lawton, Beddington and Bonser, 1974; Oaten and Murdoch, 1975a).

While predation theory has been concerned with foraging strategies associated with maximization of energy gain, theories of attack strategies by insect parasitoids are usually concerned with maximization of the number of hosts parasitized. Optimal foraging in this situation is the way in which insect parasitoids search out their hosts and respond either to changes in the distribution or density of the host population or to changes in their own density (Cook and Hubbard, 1977; Hubbard and Cook, 1978; May, 1978; Comins and Hassell, 1979; Waage, 1979).

In the classic works of Thompson (1924), Nicholson (1933) and Nicholson and Bailey (1935), mathematical models were developed to describe the interactions of predator/prey and host/parasite populations. These models estimated the fluctuations often observed in predator/prey and host/parasite numbers. From the general works on predation and insect parasitism (Holling, 1959a, 1959b, 1961; Hassell, 1966; Royama, 1971) mathematical models have been constructed to describe some of the factors which contribute to the stability of the predator/prey and host/parasite interactions (Hassell, 1971a, 1975; Hassell and May, 1973; May, 1973, 1978; Rogers and Hubbard, 1974; Murdoch and Oaten, 1975; Hassell, Lawton and Beddington, 1976; Beddington, Hassell and Lawton, 1976; Murdoch, 1977).

It is now accepted that three basic responses exhibited by predators and insect parasitoids can influence the course of the predator/prey and host/parasitoid interaction and consequently affect the stability of the relationship. These basic responses are:-

(1) <u>The Functional Response</u> (i.e. the response to host/prey density)

which describes the way in which the number of prey or hosts attacked by predators/parasitoids reaches an upper limit as the prey/host density increases (Holling, 1959a, 1965, 1966; Murdoch, 1970, 1973; Royama, 1971; Rogers, 1972, Hassell and May, 1973; Murdoch and Oaten, 1975; Oaten and Murdoch, 1975a, 1975b; Hassell, Lawton and Beddington, 1977).

(2) <u>The Aggregative Response</u> (i.e. the response to host/prey distribution)

which describes how predators and parasitoids respond in particular to areas of high host/prey distribution. Such host/prey aggregations may cause an increase in the percentage predation or parasitism (Hassell and May, 1973, 1974; Murdie and Hassell, 1973; Krebs, Ryan and Charnov, 1974; Murdoch and Oaten, 1975; Murdoch, 1977).

(3) Interference (i.e. the response to predator/parasitoid density)

which describes how increases in predator/parasitoid density reduces the searching efficiency of individual predators and parasitoids (Watt, 1959; Griffiths and Holling, 1969; Hassell and Varley, 1969; Hassell, 1971b; Hassell and Rogers, 1972; Hassell and May, 1973; Rogers and Hassell, 1974; Rogers and Hubbard, 1974; Beddington, 1975; Broadhead and Cheke, 1975; Free, Beddington and Lawton, 1977).

While a great deal of information is available for predator/prey and host/parasitoid interactions, it is only in recent years that the mathematical approach has been applied to the dynamics of host/parasite relationships,other than insect parasitoids (Crofton, 1971a, 1971b; Anderson, 1974, 1976a, 1976b, 1976c, 1978a, 1978b; Bradley, 1974; Kennedy, 1975a, 1977; May,1977; Anderson and May, 1978, 1979; May and Anderson, 1978).

One of the major problems inherent in the analysis of host/parasite interactions is caused by the frequent complexity of the parasite life cycle. As pointed out by Esch et al. (1977), there is a strong tendency to consider the parasite population biology in terms of specific isolated life cycle stages without regard to other life cycle stages of the same parasite species which may also be present in the same ecosystem. However, recent studies have discussed these problems (Anderson, 1974; Anderson, Whitfield and Mills, 1977).

Theoretical models have been constructed to analyse the regulation and stability of host/parasite population interactions in detail (Anderson, 1978a; Anderson and May, 1978; May and Anderson,1978). Essentially, the models predict that three types of population processes are significant in stabilizing the dynamics of the host/parasite interaction, while three other processes act as a destabilizing influence.

The models determine that the regulation of many host/parasite interactions is often caused by the interrelationship of both stabilizing and destabilizing processes. In general terms, the basic difference between parasites and predators or parasitoids is that parasites do not kill their host as a prerequisite for successful development. Although this difference is apparent, the three stabilizing processes which may regulate host/parasite interactions are somewhat analagous to the three processes previously mentioned (viz. functional, aggregative and interference responses) which influence the outcome of predator/prey and host/parasitoid interactions (see Anderson and May, 1978).

The dynamics of many fish host/parasite populations are quite well documented (see Kennedy, 1977, for a recent review). In very few cases however, has it been possible to demonstrate critically the existence of density-dependent factors capable of regulating these parasite populations by means of negative feedback controls. Density-dependent factors which have been described usually result from three possible processes:-

(1) Host death due to heavy parasite infections caused by overdispersion of parasites (Crofton, 1971b; Pennycuick, 1971; Boxshall, 1974a; Lester 1977).

(2) Immunological or cellular response by the fish host caused by heavy or previous parasitic infection (Paperna, 1963, 1964; Lester, 1972; Hines and Spira, 1973, 1974; Lester and Adams, 1974a, 1974b).

(3) Inter- or intra- specific competition on the fish host between parasites at high densities (Paperna, 1964; Mills, Anderson and Whitfield, 1979).

The present study is concerned with some non-specific temporary fish ectoparasites,namely the branchiuran Argulus foliaceus Linnaeus,

1758; the leeches Piscicola geometra Linnaeus, 1761, and Hemiclepsis marginata O.F.Muller, 1774, and the glochidia larvae of the fresh-water bivalve Anodonta cygnea Linnaeus, 1758. The first three species may be considered as intermediates between parasites and predators.In these organisms, parasitism exists during the foraging period with most other biological activities being carried out in the free living phase. In A.cygnea, parasitism is restricted to the larval phase only.

Little work has investigated the infection strategies of temporary fish ectoparasites. It was considered that these organisms could be investigated in terms of foraging theory since they possess certain characteristics which enable their searching and parasitic activities to be practically analysed.

II. MATERIALS AND METHODS

1. Sampling procedure

Free living specimens of Piscicola geometra and Hemiclepsis marginata were initially obtained from the River Stour at Flatford Mill, Suffolk. (O.S.Reference Sheet 168, grid reference 077 333) from October 1975- October 1976. Weed samples from the middle section of the river were removed by means of a grapple and placed in an enamel tray with river water. These samples contained P.geometra only. H.marginata, by comparison, was principally restricted to the leaf bases and axils of Typha latifolia (L.) found on the margin of the river.

Owing to the small size of the samples obtained and to difficulties encountered in the sampling of fish in this area, sampling was continued at the Lea Bridge Reservoirs, Tottenham, London. (O.S.Reference Sheet 177, grid reference 358 868). Samples of Gasterosteus aculeatus (L.) infected with Argulus foliaceus and Piscicola geometra were obtained from a single locality in the channel section of the reservoir. Uninfected fish and free living samples of parasites were also taken from the same area.

Initially, fish samples were collected with a two metre beam trawl, but on investigation it was found that many of the parasites were lost from the sample as the net was removed from the water. For quantitative monthly sampling from August 1977-July 1978, an alternative method was therefore used. A weighted plankton net (diameter at top, 35 cm.) was suspended in the water until a shoal of sticklebacks swam over the opening. The plankton net was then quickly lifted out of the water and the fish sample immediately placed in a white enamel tray containing river water. In the majority of monthly samples, Argulus would leave

fish once the sample was placed in the tray or would at least move from their initial position on the fish. Argulus or Piscicola on the fish were removed with a camel hair brush and the total number of parasites and fish in the sample were recorded. At least 30 fish were sampled every month with the exception of March, 1978, when no fish were available.

Specimens of Argulus were designated as immature, male or female estimated by the absence or presence of testes or ovaries respectively. During May 1978, 98.7 % of the Argulus infection consisted entirely of larvae. These did not leave the fish or change their attachment site on capture. Estimates were therefore made of the frequency distribution, site selection and occurrence of Argulus in different sexes and size groups of G.aculeatus for this month. The sex of the fish was determined by the distinct red colouration of the males. Samples of uninfected fish were transported to the laboratory in bins containing reservoir water and subjected to as little handling as possible. Parasites were transported to the laboratory in jars containing reservoir water.

Four monthly attempts were made to sample free living Argulus. A plankton net (diameter at top - 35 cm.) was suspended in the water and dragged through the sampling area for 15 minutes. In each sample, insignificant numbers (less than five) were obtained, consequently this method was abandoned.

Monthly samples of free living P.geometra were taken from plants growing in the middle section of the channel at Lea Bridge. Weed samples were removed by means of a grapple until approximately half a dustbin was filled. Due to the problems involved in obtaining weed in the winter months, the sampling programme was modified to ensure that at least 30 leeches were collected per month, with the exception of March when no specimens were found. Because of the difficulty of obtaining weed

samples in the winter months, no quantitative measurements of the monthly changes in the population density of leeches were made. In the laboratory, leeches were removed from the weed with a camel hair brush and length frequency distributions were measured (divided into five mm. length classes). Length was measured with the leech in a relaxed position on the bottom of a tray. The minimum parasite length of a relaxed specimen was the statistic recorded.

Specimens of adult Anodonta cygnea were obtained from the River Roding, near Chipping Ongar, Essex. (O.S.Reference Sheet 167, grid reference 564 020) in January 1977, 1978. At this point, the river is shallow with a sand/gravel bed and the flow is fairly rapid. A.cygnea were collected by hand and transported to the laboratory in plastic buckets containing river water. Several samples of fish caught with a pond net indicated that Gasterosteus aculeatus, Noemacheilus barbatulus (L.), Phoxinus phoxinus (L.) and Cottus gobio (L.) from the same habitat were infected with glochidia. Uninfected specimens of these fish were taken to the laboratory in plastic buckets containing river water.

Samples of Carassius carassius (L.) were obtained from a pond near the Queen Mary College field station at Dytchleys, Essex. (O.S. Reference Sheet 167, grid reference 551 957). Fish were sampled using a kennel trap (diameter 90cm.) baited with bread. The trap was left in the pond for several hours. Fish were removed with the trap, placed in bins containing pond water and transported to the laboratory.

2. Laboratory maintenance

Separate stocks of uninfected G.aculeatus, N.barbatulus, P.phoxinus and C.carassius and infected G.aculeatus and C.carassius were kept in constant temperature rooms, acclimatized to 10 °C. and kept on an

alternating 12/12 light/dark photoperiod. Stocks of C.gobio, Carassius auratus (L.), Gobio gobio (L.), Pungitius pungitius (L.), Scardinius erythrophthalmus (L.) and Anguilla anguilla (L.) obtained from several sources were subjected to the same conditions. Any fish exposed to parasites during the experimental period were maintained afterwards in separate tanks. All tanks containing uninfected fish were of 250 litre capacity, whereas infected fish were kept in 10 or 20 litre capacity perspex tanks. Unless otherwise stated, all tanks contained tap water, stone and weed substrate and were continually aerated. Preliminary investigations indicated that infected fish could exhibit a strong behavioural response to parasites and would occasionally escape from the tanks. All tanks were therefore covered with wire mesh.

Fish were fed weekly on Tubifex tubifex except for Carassius carassius, Carassius auratus and Scardinius erythrophthalmus which were fed daily on a propietary fish food. All fish to be used in the experiments were free from other obvious parasitic infection and had not previously been exposed to ectoparasites in the laboratory. Sticklebacks used in the experiments were approximately 25 - 50 mm. in length, minnows 80 - 100 mm., stone loach 90 - 110 mm. and crucian carp 100 - 120 mm. in length.

A.foliaceus were maintained on either G.aculeatus or C.carassius. Approximately 60 G.aculeatus were exposed to immature (i.e. no visible sign of well developed gonads) Argulus in a plastic tank of 250 litre capacity. Five batches of five C.carassius were similarly exposed and kept in separate 10 litre capacity tanks. All tanks were filled with tap water, constantly aerated, kept at ambient laboratory temperature (approximately 18 - 20 ^{o} C.) and an alternating 12/12 light/dark photoperiod and covered with wire mesh to prevent escape of fish.

Tanks were cleaned weekly and any dead fish replaced. Fish were fed as previously mentioned. Mature, female Argulus deposited eggs on the tank walls and hatching usually occurred after four to six weeks. Batches of larvae to be used in experiments were removed daily with a pasteur pipette, while the remainder were left as permanent stock. Argulus obtained from monthly field sampling were used for life cycle maintenance. When mature, Argulus to be used in experiments were removed from the fish with a camel hair brush.

Stocks of P.geometra were maintained either at 10 or 20°C. in plastic tanks containing 20 litres of tap water, stone and weed substrate and constantly aerated. The same conditions as described above for Argulus were employed. Leeches were allowed to feed every two to three weeks on crucian carp at the prescribed temperatures. Several days after copulation of the leeches, cocoons were deposited either on the stones, weed or tank walls. These hatched approximately three to four weeks later at 20°C., or eight to ten weeks later at 10°C. Batches of recently hatched leeches and adults were removed when required for experiments. The remainder were maintained as permanent stock. Immature leeches were left in stock and were not used in experiments until they were mature (approximately 20 - 22 mm. in length).

Stocks of H.marginata were difficult to maintain in the laboratory. Attempts were made to maintain leeches using the same techniques and conditions as for P.geometra. Although breeding did not occur in the laboratory, mature wild leeches produced eggs which hatched after approximately 15 - 23 days at 20°C. Young leeches were cared for on the ventral body surface of the adult leech until the first feed. Although leeches readily fed, they were subjected to heavy predation by their fish hosts. This fact, plus the difficulties of

establishing a breeding colony meant that only initial observations on infection strategies were possible.

Specimens of adult Anodonta cygnea were maintained in aerated tap water in 20 litre capacity tanks and kept at ambient laboratory temperature (approximately 18 - 20°C.). Glochidia larvae were normally produced several days after collection and daily batches were removed for experimentation. When glochidia were discharged from the adult bivalve, they were generally connected together by byssus threads. All larvae used in the experiments were therefore individually separated with a pasteur pipette. Only larvae with their shell valves open were used and unless otherwise stated were one day old.

3. Experimental procedure.

The investigation of infection considered the initial phase of attack, rates of attack, effects of host and parasite density, distribution and site selection. Individual experimental procedures are described below.

(a) First attack

Initial attack and attachment to fish was investigated in the following manner:- Argulus and Piscicola adults were removed from stock cultures at 20°C. and exposed to host fish at 10°C. These exposed fish were maintained using the same techniques and conditions as described above for at least two weeks prior to experiments.

Preliminary investigation indicated that a starvation period of one day for Argulus and one week for Piscicola was required before parasites would attack fish. Consequently, before the start of each experiment, Argulus were removed from the infected sticklebacks and starved for one day, whereas Piscicola were removed from crucian carp and starved for one week. Infective Argulus were separated into batches of mature

females (approximately 6 mm. x 3 mm.) and mature males (approximately 5 mm. x 2 mm.). All infective Piscicola were 20-30 mm. in length.

Experimental tanks measured 30 x 20 x 20 cm. and contained 10 litres of tap water at 10 ^{o} C. Tanks used for leeches contained weed substrate. All infection trials were started at the same time of day.

The time taken for the first attack to occur at different parasite densities was determined for Argulus and Piscicola. First attack was assumed once an individual fish was parasitised by an individual parasite. Subsequent infection was ignored in this particular experiment. Separate populations of male and female Argulus were used, partly to investigate possible differences in the searching efficiency of the two sexes and partly to avoid copulation which could affect the searching potential of the parasites.

The selected attacking density of Argulus was pipetted into a tank, while leeches were placed in a tank with a camel hair brush. Parasites were allowed to acclimatize to tank conditions for ten minutes. A previously uninfected stickleback was introduced into the tank and observations were made at ten minute intervals, or in the case of higher densities at one minute or one second intervals, to determine the first successful attack. This was recorded once a parasite remained attached to the fish for at least ten seconds. When the fish had been attacked for the first time, it was removed from the tank together with any attached parasites and neither were used again. Fifteen trials, that is 15 exposures and first attacks were carried out at each density of parasites. This theoretically represents a population of 15 fish being separately exposed to a given density of parasites. When increased densities of sticklebacks were used per tank, the time taken for the first attack on the first fish only was recorded. In order to standardise

the procedure, the experiments were terminated at each density when ten time intervals (i.e. 0 - 10 seconds, 0 - 10 minutes or 0 - 100 minutes) were completed.

The densities of P.geometra used were 5, 10, 30 and 50 exposed to one G.aculeatus per tank (representing a total of 60 trials). The experimental regime for different densities and sex of A.foliaceus and different densities and species of fish is given in Table 1 (representing a total of 345 trials). The numbers given indicate the density of fish or parasites per tank.

(b) Infection pattern

This series of experiments were designed to investigate the infection pattern typical of male and female A.foliaceus and P.geometra onto fish. The experimental procedure and conditions were the same as in II.3 (a), except leeches were starved for either one or two weeks before being exposed to fish in tanks of 20 litre capacity (60 x 30 x 15 cm.) both with and without substrate. Each trial lasted for 12 hours with Argulus and either 10 or 100 minutes with Piscicola. Readings were taken at 30 minute, 30 second or 10 minute intervals respectively to determine the number of attached parasites on any one fish. The experimental regime for different host species, densities and sex of A.foliaceus and also for 20 P.geometra with different starvation periods, substrate and host species are given in Tables 2 and 3 respectively. 50 trials were completed for Argulus and 25 for Piscicola.

(c) Attack rate

In order to examine the precise nature of the infection process as a consequence of II.3 (a) and (b), further experiments were designed to investigate the relationship between successive attacks during infection. Argulus used in these experiments were subjected to the same

Table 1

First attack by Argulus foliaceus

Argulus density (female)	Host density of G.aculeatus	Argulus density (male)	Host density and species 1	2	3	4
5	1	5	1			
10	1	10	1			
15	1	15	1			
20	1,2,3,4,5	20	1			
25	1	25	1			
30	1	30	1	1	1	1
35	1					
40	1					
45	1					
50	1					

Host species

1 = Gasterosteus aculeatus

2 = Phoxinus phoxinus

3 = Noemacheilus barbatulus

4 = Carassius carassius

Table 2

Infection pattern by Argulus foliaceus

Argulus density (female)	Host density of G.aculeatus	Argulus density (male)	Host species and density 1	2	3	4
5	1	5	1			
10	1	10	1			
20	1	20	1	1	1	1
50	1					

Host species

1 = Gasterosteus aculeatus

2 = Phoxinus phoxinus

3 = Noemacheilus barbatulus

4 = Carassius carassius

Table 3

Infection pattern by Piscicola geometra

Substrate	Host species and starvation period (weeks) of leeches C.carassius	N.barbatulus
with	(1, 2)	-
without	(1, 2)	(1)

conditions as in II.3 (a). One stickleback was exposed to 50 female Argulus and the time taken for successive attacks to occur up until the fifth attack, was continuously recorded. Fifteen trials were completed.

(d) Effect of parasite and host density on infection

Argulus adults were maintained on sticklebacks as described above (II.2). Batches of Argulus larvae were removed from 20 ^{o} C. with a pasteur pipette at daily intervals and acclimated at 10 ^{o} C. Since the searching capacity of the larvae may alter with time, larvae of approximately one day old were used. Fish were treated as in II.3 (a).

An initial experiment was carried out with one stickleback being exposed to different densities of larvae in 500 ml. of water at 10 ^{o} C. to estimate the number of larvae attached to fish after ten minutes. Twenty trials were completed. This experiment suggested the use of greater volumes of water, mainly to increase the available area for host and parasite movement. Consequently, further experiments were carried out in plastic tanks (30 x 20 x 20 cm.) all containing two and a half litres of water at 10 ^{o} C. Unfortunately, lack of experimental material due to heavy predation and natural mortality of larvae did not allow for replication of the initial experiment in two and a half litres of water.

The densities of adult and larval Argulus used were 2, 4, 8, 16, 32 and 64. These numbers were exposed to stickleback densities of 1, 2, 4 and 8 in two and a half litres of water (with the exception of larval densities 2 - 64 being exposed to one G.aculeatus in 500 ml. of water). Where the numbers of fish exceeded the number of parasites, no experiments were completed. In order to maintain similar nutritional status between adults and larvae, adults were starved for one day prior to exposure. All experiments at any given ratio of parasite/host densities were repeated

20 times. The appropriate density of Argulus was pipetted into the tank and previously uninfected fish were exposed to the parasites for ten minutes. After this time, the fish were removed and examined to estimate the number of attached Argulus. A total of 840 trials were completed at all densities of Argulus adults and larvae with sticklebacks.

Densities of 2, 5, 10 and 20 Piscicola, starved for one week, were separately exposed to one crucian carp in 20 litres of water (tanks were 60 x 30 x 15 cm.) at 10°C. Leeches were placed on the fish with a camel hair brush and it was observed that all leeches were in the feeding position after one hour. To investigate the effect of density on feeding, readings were taken at daily intervals to estimate the numbers of leeches remaining on the fish or which had fed and subsequently left the host. Fed leeches were a distinct blood red colour(as opposed to starved leeches, which were green) and relaxed on the walls of the tank. Fed leeches were removed to avoid predation by the fish. Five trials were completed at each density and the experiment was continued until all leeches had left their respective hosts.

(e) Distribution and site selection

i. Dispersion pattern

These experiments were designed to investigate the frequency distribution of Argulus and glochidia larvae when a population of sticklebacks were exposed to a single wave of infection. 150 Argulus larvae and 500 glochidia larvae were individually pipetted into separate plastic tanks (60 x 30 x 15 cm.) containing 20 litres of water at 10°C. Larvae were approximately one day old. In the case of glochidia, the water was gently agitated to ensure an even suspension of parasites. Either 30 or 50 fish were then added to the tanks and exposed to the Argulus or glochidia larvae for one and three days respectively. Fish

were individually removed with a minimum of disturbance to settled larvae and the number of attached larvae (per fish) was determined. Fish were then killed by pithing and dissected under a binocular microscope to estimate the numbers of parasites attached to the gills and buccal cavity.

ii. Initial attachment sites

These experiments investigated the site distribution of recently hatched parasites. 20 one day old Argulus larvae and 100 one day old glochidia were pipetted into each of 30 beakers containing 500 ml. of water at 10°C. Individual sticklebacks of approximately the same size were added to each beaker and exposed to the larvae for 24 hours. Fish were then removed and examined for site distribution as described above (II.3. (e)i.).

A similar experiment was designed to study the site distribution and feeding areas of recently hatched Piscicola upon crucian carp. Because many of the young leeches were preyed upon by fish, another experiment was designed to investigate site distributions amongst adults. 100 Piscicola which had been starved for one week were removed from the stock tank and put into a plastic tank containing 50 litres of water at 10°C. The leeches were allowed to acclimatize to tank conditions for 30 minutes. 25 crucian carp were then added to the tank and exposed to the leeches for ten minutes. Any leeches which had not attached after this time were removed and replaced in stock. Fish were removed separately and examined for attachment sites. Since many of the leeches were in the process of feeding, the positions of both the anterior and posterior suckers were noted.

In order to describe the positions of attached parasites, the body surface of the fish was arbitarily divided into three areas (anterior, middle and posterior) by two vertical lines. (For glochidia, a further

horizontal line running along the lateral line was also used). In the stickleback, vertical lines run ventrally from the anterior edge of the first dorsal spine parallel to the anterior edge of the pectoral fin and from the anterior edge of the dorsal fin to the anterior edge of the anal fin. Positions of larvae on the left or right sides (for Argulus) or ventral and dorsal regions (for glochidia) of the body, fins and spines were noted. Attachment to fin bases were included in the fin totals. Internal positions of glochidia larvae were considered as being attached to the buccal cavity or to the gills. These were separated into 1st., 2nd., 3rd. or 4th. gill arches plus filaments. In crucian carp, the body surface of the fish was similarly divided into three areas with vertical lines running from the anterior edge of the dorsal fin to the posterior position of the pectoral fin and from the posterior edge of the dorsal fin to the anterior edge of the anal fin. Positions of both suckers were noted for attached leeches.

iii. Change in attachment sites

Experiments were designed to study the possible changes in site distribution of larval ectoparasites during development as a function of temperature and host species.

At 10 ^{o} C. and 20 ^{o} C., 50 one day old Argulus larvae were exposed to five crucian carp and five sticklebacks. Similarly, 100 glochidia were exposed to five sticklebacks. At each temperature, fish were exposed to larvae in plastic tanks (30 x 20 x 20 cm.) containing 10 litres of water. Tanks at 20 ^{o} C. were placed inside a Grant model (constant temperature) circulating water bath and the light regime was kept as constant as possible within the constraints of the laboratory. Infection experiments at 10 ^{o} C. were carried out in constant temperature rooms (II. 2).

The site selection of Argulus and glochidia larvae was noted after 24 hours and subsequently at weekly intervals until the parasites were mature. In some cases, death of fish or parasites terminated observations prematurely. Attachment sites were analysed as described in II.3 (e)i. No account was taken of the varying numbers of parasites on each fish.

Another experiment investigated the possible movement of Argulus between fish. Ten three week old Argulus were exposed to one crucian carp in a small dish containing 500 ml. of water at 20°C. This was repeated four times. All Argulus initially remained attached to the fish for at least two hours. Fish were then placed in a plastic tank (60 x 30 x 15 cm.) containing 20 litres of water. Fish were individually separated with a netlon partition of similar dimensions to the tank, which allowed for any possible movement to Argulus between fish. The total numbers of Argulus attached per fish were monitored daily for one week.

To study possible changes in the site distribution of Piscicola on crucian carp, the experiment for adult leeches described in II.3(e)ii. was continued. The experiment was monitored for 116 hours in order to estimate changes in feeding sites and the length of time spent on the fish. Positions of leeches on the 25 fish were noted at 20, 44, 68, 92 and 116 hours. The number of leeches successfully fed and no longer attached to the fish were also recorded. In order to analyse changes occurring on individual fish, four plastic tanks (30 x 20 x 20 cm.) each containing six fish in 10 litres of water were used. The initial positions and numbers of leeches were used as markers for individual fish. In this way, no confusion arose as to changes in the numbers or feeding positions of leeches on individual fish. Leeches which had

completed feeding were removed from the tanks.

(f) Growth, survival and effect of ageing on infectivity

Estimates of growth and survival of Argulus on fish hosts were determined from the experiments described above (II.3.(e)iii.).

The survival of free living young Argulus and leeches was estimated as follows: 50, one day old Argulus larvae and 30, one day old Piscicola were acclimated at 10°C. Parasites were studied in aerated 500ml. beakers containing 250 ml. of water. The number of larvae and young leeches surviving at daily intervals was recorded until all had died. Death was determined if there was no response to manual stimulation by a seeker, monitored under a binocular microscope.

Since young Piscicola were particularly susceptible to predation, an experiment was conducted to eliminate this factor, in order that growth could be more accurately assessed. One crucian carp was suspended in a netlon cage (designed to restrict movement of the fish) and placed inside a 10 litre perspex tank at 20°C. The tank was placed inside a water bath as described above (II.3.(e)iii.). The fish was exposed to 30, one day old leeches which were allowed to feed until satiated. Having fed, leeches would detach from the fish and relax on the tank walls. Leeches were removed from the tank, kept at 20°C., starved for one day and then measured. Length measurements were taken from the tip of the anterior sucker to the top of the posterior sucker, once leeches were in a relaxed position on the bottom of a petri dish. Leeches were allowed to feed until they reached maturity. This was assumed once the first cocoons had been laid in the tank. At each feed, leeches were placed with another previously uninfected fish, then removed and measured as described.

A further method was employed to measure the survival of adult Piscicola during starvation. All leeches were approximately 20 - 30 mm.

in length. One crucian carp was exposed to 30 Piscicola, both at 10°C. and at 20°C. In order to simulate previous nutritional status, leeches were starved for two weeks and then allowed to feed on the crucian carp for 24 hours. Leeches were then isolated and the numbers of leeches surviving each week were estimated.

Freshly shed glochidia were retained for 24 hour periods prior to exposure to sticklebacks. A batch of 50 glochidia were exposed to a single fish at 10°C. in a 500 ml. beaker containing 100 ml. of water. This was repeated nine times. After 24 hours, the fish were removed and the number of attached larvae counted as described previously (II.3.(e)i). This procedure was carried out at daily intervals for six days in order to assess the influence of glochidial ageing on infectivity.

(g) Host species

Different host species were exposed to P.geometra and H.marginata at 20°C. The experimental procedure was the same as for leeches described in II.3.(e)iii., with the addition of stone and weed substrate. Ten starved leeches were introduced into the tank and allowed to acclimate for 24 hours, after which a fish was introduced. Each fish was observed for a period of several hours to note the method of attack by leeches and whether feeding occurred. The species principally used were G.aculeatus, C.auratus, N.barbatulus and G.gobio. Other fish species, namely P.phoxinus, C.carassius, P.pungitius, C.gobio, A.anguilla and S.erythrophthalmus were also separately exposed to leeches over a period of several days to note whether feeding occurred.

Different host species were similarly exposed to Argulus to observe if attack and feeding occurred.

4. Statistical analysis

An important section of the present work is concerned with the phenomenon of first attack. In order to analyse the data obtained, probit analysis was used (Finney, 1971). Since this type of analysis is rarely used in parasitological problems, it will be described below in some detail.

Probit analysis provides a set of statistical techniques used in many biological situations for considering the relationship between the frequency distribution of a reaction in relation to a stimulus that produces an all-or-nothing response (i.e. quantal response). It was considered that probit analysis could be applied to the host/parasite system, when first attack by an ectoparasite occurs on an individual fish. In this sense, the quantal response (i.e. parasitism) occurs when one fish in the population is attacked for the first time and where the stimulus (i.e. attacking parasites) could be applied as a quantitative measure (dose) by varying the density of parasites or the time to which the fish are exposed to the parasites. Hence the quantal response is simply distinguished by parasitised or non-parasitised fish, where the proportion of parasitised to non-parasitised fish in the population at any one time will be dependent on the stimulus. For any one fish in the population exposed to parasites for the first time, there will be a certain level of parasite intensity (measured by the density of parasites in conjunction with the exposure time) below which parasitism is unlikely to occur in measurable time and above which it does occur, this value is the " system tolerance ". Tolerance will vary from fish to fish and will be partly controlled by inherent differences in the susceptibility of the fish to attack and the difference in attack potential of individual parasites. If the intensity of the stimulus is measured by z, the distribution of

tolerances is expressed by:-

$$dP = f(z)\, dz \qquad (1)$$

which is the proportion, dP, of the host population whose tolerances lie between z and dz at the time of testing, where dz represents a small interval on the dose scale. The factor relating dP to the length of this interval is the frequency function, f (z), determined for all values of z.

If a dose z_o is given to the whole population, every fish with a tolerance less than z_o would become parasitised. The proportion of parasitised fish is P, where

$$P = \int_o^{z_o} f(z)\, dz \qquad (2)$$

The measure of the dose may be a quantity ranging from 0 to infinity, but the response would be positive at any dose, except 0, (given a long enough time exposure) so that

$$\int_o^{\infty} f(z)\, dz = 1 \qquad (3)$$

This yields a frequency distribution which is typically skewed.

Normalization can be effected by considering the tolerances in terms of log time. The tolerances are now normally distributed and the log dose metameter is given by x . Every fish whose tolerance to attack by a fixed density of parasites is less than a stated value of x (log time of exposure) will become parasitised. A graph of percentage parasitism amongst the fish population (represented by the percentage of fish attacked for the first time at any given density of parasite) against x (= time of exposure) will be of a normal sigmoid form (see Figure 6). The normal frequency distribution of log tolerances as in (1) written in terms of the dose metameter is then given by:-

$$dP = \frac{1}{\sigma\sqrt{(2\pi)}} \exp \left[- \frac{(x - \mu^2)}{2\sigma^2} \right] dx \qquad (4)$$

where the parameters μ , = the mean and σ, the standard deviation.

On this basis, given a population of previously uninfected fish

being exposed to ectoparasites, the probability P, that one fish when exposed (for a certain time at a fixed density), will be parasitised for the first time, is represented by the proportion of fish that would be parasitised if the whole population received that dose.

This is given by:-

$$P = \int_{o}^{z_o} f(z)\, dz \qquad (2)$$

The probability of not being parasitised will be given by (1 - P) or Q. This generates a binomial distribution, whereby if a population of n fish is exposed to parasites and if all hosts and parasites react independently, then the probabilities of n, (n - 1), (n - 2), ..., being attacked, are the (n + 1) terms in the expansion of $(P + Q)^n$. The probability of r being attacked is:-

$$Pr\ (r/n) = \frac{n!}{r!\ (n - r)!}\ P^r Q^{n - r} \qquad (5)$$

giving the binomial expansion of probabilities. If different batches of fish are exposed to a dose of parasites, the average number of attacked fish per batch will be n/P. By controlling the factors likely to affect the dependency of attack in the laboratory, the differences in the susceptibility of the fish will be manifested. By testing a batch of fish at ten fixed time intervals, using time as a series of doses at any fixed density of parasite, then for each dose, a proportion

$$p = r/n$$

of fish will become parasitised and will therefore have a tolerance less than the given dose. Each p will be an estimate of P, namely the proportion in the fish population of which the batch is a sample. Dose as time was administered in ten minute intervals at low densities, or at one minute or one second intervals at high densities of parasites, considering the time intervals to be discrete and not continuous.

Analysis by probit will give for any distribution of tolerances

an estimate of the dose (time) required to produce infection for the first time in fifty percent of the fish population at any given density of parasite. For any distribution of tolerances, the ED 50 is the value of z_o that in equation (2) gives

$$P = 0.5$$

Thus, equation (2) can be solved if the parameters in the function f (z) are known. The corresponding value of the dose metameter x is equivalent, thus, in equation (4), the log ED 50 is the value of x_o where

$$0.5 = \int_{-\infty}^{x_o} \frac{1}{\sigma\sqrt{(2\pi)}} \exp\left[-\frac{(x-\mu^2)}{2\sigma^2}\right] dx \qquad (6)$$

which gives the mean μ . Here the tolerance distribution is symmetrical about the mean μ ,consequently, the ED 50 is equal to the mean and median of the distribution on the x scale of dose measurement.

By using probit analysis, it is possible to compare the resulting frequency distributions obtained at each density of parasite when first attack in a population of fish occurs. Probit therefore incorporates the concept of variation in individual fish to attack and allows for the way in which parasite density affects the outcome of infection to be investigated.

The mathematical model used for analysing infection rates of miracidia onto snail hosts has been discussed by Anderson (1978b). It was considered that this basic model could be used to analyse infection rates of A.foliaceus and P.geometra and will therefore be described below. Further reference to this model will be discussed later (page 46).

If the chance of any given Argulus infecting a fish in the interval of time Δt is $\propto \Delta t$ then the net rate at which a single host becomes infected when exposed to a population of Argulus of size M (t) is $\propto M(t)\,\Delta t$. It is assumed that the instantaneuous rate of infection $\propto$ is a constant for each Argulus in the population (M (t)) and for each fish to infective parasites. The rate is defined as Argulus/fish/unit of time.

If P (t) represents the number of Argulus which have infected a single host by time t, the rates of change M (t) and P (t) with respect to time can be expressed as:-

$$\frac{\delta Mt}{t} = - \alpha M(t) \qquad (7)$$

$$\frac{\delta Pt}{t} = \alpha M(t) \qquad (8)$$

If the infection experiment is started at time t = 0, by exposing the uninfected host (P (o) = 0) to M_o Argulus then (7) and (8) can be expressed as:-

$$M(t) = M_o \exp(-\alpha t) \qquad (9)$$

$$P(t) = M_o (1 - \exp(-\alpha t)) \qquad (10)$$

As the duration of fish exposure to infection (t becomes large) increases, the pool of free swimming Argulus (M (t)) tends to zero and conversly, the number of Argulus infections P (t) rises gradually to M_o.

Applying the same rationale to the infection process onto a single host, the mathematical model can be extended to consider the effect of increasing host density (Anderson, 1978b). However, this model can only be used if the infection rate (α) is constant onto a single host. If increasing host density does not alter parasite behaviour then the net rate of infection within a population of S fish, when exposed to M (t) Argulus will be α S M (t) Δ t, in the time interval Δ t. Equations (9) and (10) become:-

$$M(t) = M_o \exp(-\alpha S t) \qquad (11)$$

$$P(t) = M_o - M(t) \qquad (12)$$

where P (t) represents the total number of infections in the population of S fish. The mean number of infections/fish is P (t)/S at time t.

Statistical analysis for other experiments was determined by techniques described in Bailey (1959), Snedecor and Cochran (1967) and Elliott (1971).

III. Argulus foliaceus

Introduction

Amongst the Crustacean parasites of fish, some three major groups are now recognised:- the Branchiura, Copepoda and Isopoda (Kabata, 1970). The present study is concerned with one representative species of the Branchiura, namely Argulus foliaceus.

The Branchiura consists of some 130 species (Yamaguti, 1963), about 100 of which belong to the genus Argulus Muller, 1785. Epizootics of fish lice often cause heavy mortality in fisheries (Schumacher, 1952; Allum and Hugghins, 1959; D'Ancona, 1960; Williams, 1964; Bazal, Lucky and Dyk, 1969; Kolipinski, 1969; Kroger and Guthrie, 1972; Laurent, 1975). Consequently, a great deal of work has concentrated upon finding suitable eradication methods (Sarig, 1948, 1968, 1971; Hindle, 1949; Bauer, 1958a; Saha and Sen, 1958; Sarig and Lahav, 1959; Stammer, 1959; Kelly, 1962; Lahav, Shilo and Sarig, 1962).

Only two Argulus species are commonly found in the British Isles, Argulus coregoni Thorell, 1864 and Argulus foliaceus (Gurney, 1948; Kennedy, 1974). While A.coregoni is largely restricted to oligotrophic conditions (Gurney, 1948; Mishra and Chubb, 1969; Chubb, 1970; Campbell, 1971), A.foliaceus is quite widespread and occurs throughout most of the British Isles (Kennedy, 1974).

Host records of A.foliaceus on freshwater fish indigenous to the British Isles (Maitland, 1972) are given in Table 4. Foreign records indicate that A.foliaceus is found on 28 freshwater fish species in other regions of the Palaearctic (Romanovsky, 1955; Van Duijn, 1956; Dogiel et al., 1958; Bykhovskaya-Pavlovskaya, 1964). In total, 48 fish species have been recorded as hosts for this non-specific ecto-parasite. A.foliaceus has also been reported on tadpoles of Trituris

Table 4

Host records for Argulus foliaceus

Fish species	Reference
Salmo salar (L.)	Bower-Shore (1940) Kane (1966) Kennedy (1974)
Salmo trutta (L.)	Kane(1966) Campbell (1971) Wootten (1973) Kennedy (1974, 1975b)
Salmo gairdneri (Rich.)	Wootten (1973) Kennedy (1974)
Coregonus lavaretus (L.)	Copland (1957) Kennedy (1974)
Coregonus albula (L.)	Campbell (1971) Kennedy (1974)
Esox lucius (L.)	Scott and Scott (1913) Gurney (1948) Copland (1957) Kane (1966) Mishra and Chubb (1969) Rizvi (1969) Anderson (1971) Canning et al. (1973) Kennedy (1974, 1975b) Kennedy et al. (1975)
Cyprinus carpio (L.)	Clark (1902) Scott and Scott (1913) Bower-Shore (1940) Kane (1966) Anderson (1971) Kennedy (1974) Buckley and Morrice (1976)
Carassius carassius (L.)	Anderson (1971) Kennedy (1974)
Gobio gobio (L.)	Bower-Shore (1940) Anderson (1971) Kennedy (1974) Kennedy et al. (1975)
Tinca tinca (L.)	Clark (1902) Kane (1966) Anderson (1971) Kennedy (1974)
Abramis brama (L.)	Gurney (1948) Mishra and Chubb (1969) Anderson (1971) Campbell (1971) Kennedy (1974) Kennedy et al. (1975)

(contd..)

Fish species	Reference
Scardinius erythrophthalmus (L.)	Anderson (1971) Canning et al. (1973) Kennedy (1974, 1975b) Buckley and Morrice (1976)
Rutilus rutilus (L.)	Clark (1902) Gurney (1948) Copland (1957) Williams (1964) Mishra and Chubb (1969) Rizvi (1969) Anderson (1971) Campbell (1971) Canning et al. (1973) Wootten (1973) Kennedy (1974, 1975b) Kennedy et al (1975) Buckley and Morrice (1976) Lee (1977)
Leuciscus cephalus (L.)	Kennedy et al. (1975)
Leuciscus leuciscus (L.)	Kennedy et al. (1975)
Anguilla anguilla (L.)	Kennedy et al. (1975)
Gasterosteus aculeatus (L.)	Carphin (1895) Clark (1902) Scott and Scott (1913) Bower-Shore (1938, 1940) Campbell (1971) Kennedy (1974, 1975b) Present study
Pungitius pungitius (L.)	Wootten (1973)
Perca fluviatilis (L.)	Gurney (1948) Mishra and Chubb (1969) Rizvi (1969) Anderson (1971) Canning et al. (1973) Wootten (1973) Kennedy (1974, 1975b) Lee (1977) Pickering and Willoughby (1977)
Platichthys flesus (L.)	Gurney (1948)

vulgaris vulgaris (Bower-Shore, 1940).

The life cycle of Argulus foliaceus has been discussed by a number of workers (Jurine, 1806; Clark, 1902; Wilson, 1902; Herter, 1927a; Bower-Shore, 1938, 1940; Bauer, 1958a; Kollatsch, 1959). Mating by mature adults may occur either on the fish or in the free living phase. After mating on the fish, gravid females leave the host to deposit eggs (Bauer, 1958a) on stones (preferably in shaded localities)(Herter, 1927a), or upon vegetation (Laurent, 1975). The eggs are ellipsoidal, approximately 0.35 - 0.45 mm. in length and are laid in rows of up to 653 per batch (Clark, 1902; Bower-Shore, 1940). Development usually takes from three to eight weeks, although hatching can be influenced by temperature (Jurine, 1806; Clark, 1902; Bauer, 1958a; Kollatsch, 1959). The larva is approximately 0.6 - 0.75 mm. in length and the first moult occurs after about five days. A series of moults occur with increasing size. Sexual maturity is reached after three to four weeks and A.foliaceus are 2.5 -3.0 mm. in length (Wilson, 1902).

Many active free-living larval parasites exhibit behavioural responses which increase the probability of encounters with potential hosts (Kennedy, 1975a, 1976). Herter (1927a) showed that A.foliaceus react to water currents created by the respiratory or fin movements of fish and that the parasite exhibits a positive rheotactic response in order to locate and finally infect a host. Chemoreception is only shown when A.foliaceus have attacked the host. Such unresponsiveness to chemical gradients in water has also been demonstrated for free living crustacean larvae (Crisp and Meadows, 1962) and for parasitic crustacean larvae in the free living phase (Boxshall, 1976; Kabata and Cousens, 1977).

Bazal, Lucky and Dyk (1969) found a distinct localization of A.foliaceus on the caudal and dorsal fins and sides and back of the body

on different carp species. They suggested that because the ventral surface of the carp is generally in close contact with the bottom substrate that A.foliaceus would unlikely to be found in this position. Buckley and Morrice (1976) noted several interspecific variations between the site selection of A.foliaceus on different host species.

Attachment to fish is maintained through the use of suckers (modified second maxillae), maxillipeds and hooks on the carapace. Feeding in A.foliaceus has been discussed in detail by a number of workers (Wilson, 1902; Martin, 1932; Bower-Shore, 1940; Kabata, 1970).

In recent years, A.foliaceus has been implicated as a vector of hemorrhagic septicaemia (Red disease or infectious dropsy) (Dombrowski, 1953; Bauer, 1958a; Pfeil-Putzien, 1978; Pfeil-Putzien and Baath, 1978), and also as a facultative agent of secondary infection, particularly Saprolegnia fungi (Bauer, 1958a; Pickering and Willoughby, 1977). Pathogenic effects of Argulus have been discussed by Bauer (1958b) and Kabata (1970). A.foliaceus has been recorded as an intermediate host for the nematode Molnaria erythrophthalmi (Moravec, 1978).

Aspects of the anatomy, morphology, physiology and taxonomy of A.foliaceus are well documented (Jurine, 1806; Leydig, 1850, 1886; Claus, 1875; Nettovich, 1900; Wilson, 1902; Thiele, 1904; Ivanfi, 1926; Herter, 1927a, 1927b, 1928a; Martin, 1932; Gurney, 1948; Debaisseux, 1953; Romanovsky, 1955; Stammer, 1959; Madsen, 1964; Heutschel, 1969; Czeczuga, 1971). Relatively little attention, however, has been paid to the life history and ecology of the parasite. Due to the fact that the parasite leaves the fish host during its breeding season (Wilson, 1902) or during metamorphosis (Clark, 1902), A.foliaceus has been termed a temporary ecto-parasite. Bower-Shore (1940) suggested that there was no definite breeding in A.foliaceus, while other workers (Claus, 1875, quoted by Wilson, 1902;

Clark, 1902; Bauer, 1958a; Rizvi, 1969) stated that there are three breeding seasons during the year when A.foliaceus leave the fish to deposit eggs. Reproduction ceases below 14°C. (Kiselev and Ivleva, 1953, quoted by Bauer, 1958a) and overwintered parasites begin to breed in the following spring, when an increase in water temperature occurs (Rizvi, 1969).

Factors contributing to the abundance of A.foliaceus in the free living phase or to its incidence during the parasitic phase are rarely studied. The present section comprises an analysis of some of these factors. The parasite was chosen for experimental purposes, since the ectoparasitic relationship with its fish hosts made observation relatively easy to study. Problems were also alleviated by its wide availability in the field and ease of cultivation in the laboratory.

Materials and methods

See Sections 1 - 4 (pages 7 to 21).

Results

1. Field sampling

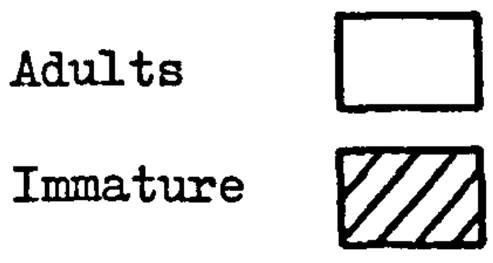
(a) Seasonal variation in occurrence

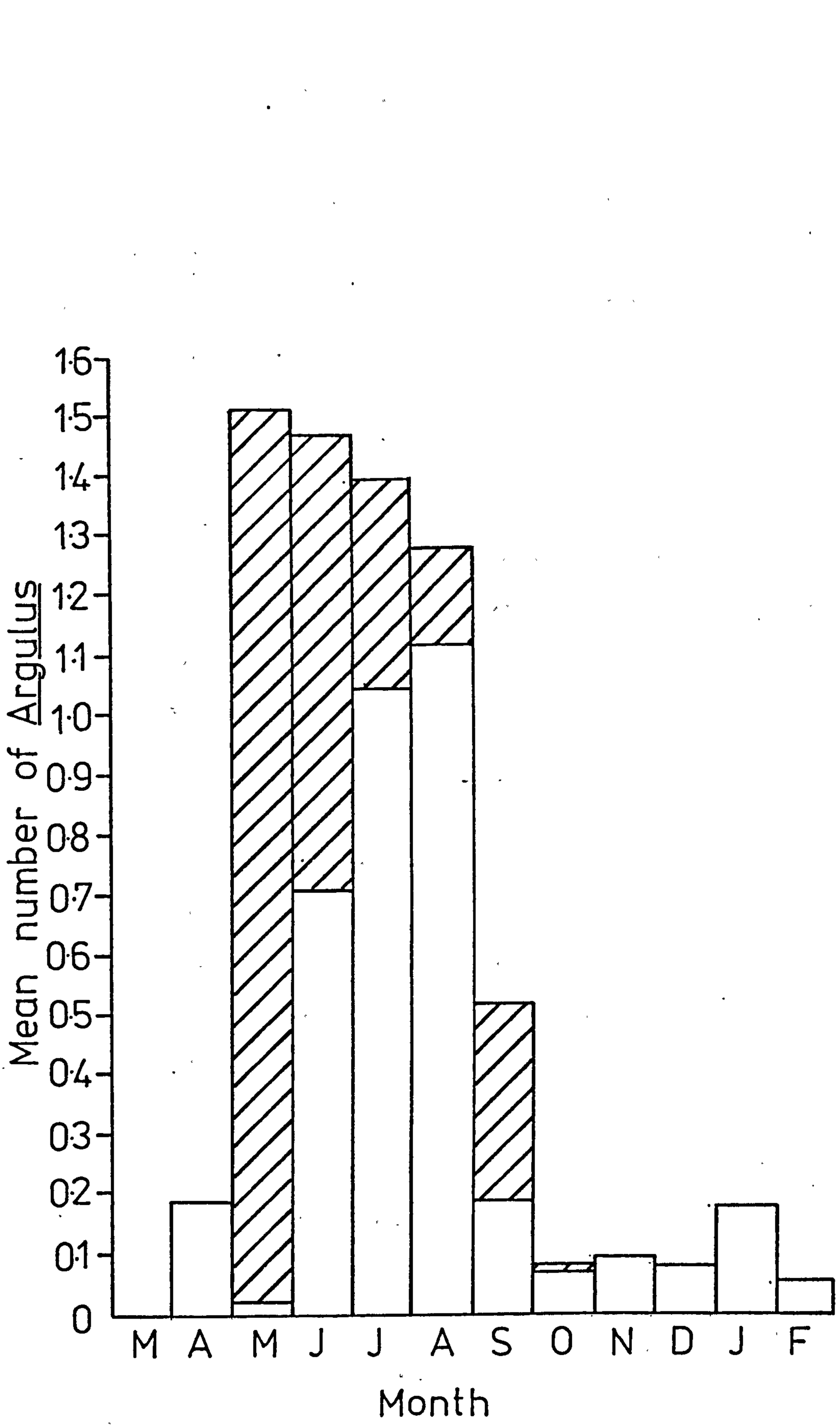
A.foliaceus occurred on G.aculeatus throughout the year (with the exception of March when no fish sample was available). The number of fish sampled per month is given in Table 17 for the incidence of P.geometra. There was a distinct seasonal variation in abundance (Figure 1). The intensity of infection was lowest in February when the mean number of parasites per fish was 0.053, while peak intensity was reached in May with a mean number of 1.51 parasites per fish.

Figure 1

Seasonal variation in the

occurrence of <u>Argulus foliaceus</u>




Mean number of Argulus
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
M A M J J A S O N D J F
Month

Growth of the population was limited to approximately four months of the year, from the end of April to August. This suggests a distinct breeding season. The age structure of the population was such that from November until April, the population consisted only of adult male and female forms. These had presumably overwintered from the previous autumn. Breeding begins in late April and in May, 98.7 % of the population consisted of immature stages. This marked change in the population structure suggests that mortality had occurred amongst the overwintering adults. Breeding continues until October.

The ratio of female to male A.foliaceus throughout the year is shown in Figure 2 . An increase in the proportion of females begins in April prior to breeding and continues until December. With the exception of February, April and May, a greater number of females were always present in the adult population.

(b) Distribution within the host population

As mentioned earlier (Section 11. 1,page 8), A.foliaceus frequently leave the fish during sampling. The presence of immature stages (which do not leave the fish or change sites so readily), provided a limited opportunity to analyse the site selection and occurrence of the parasite in different sexes and size groups of G.aculeatus.

The infection of fish with immature A.foliaceus in May 1978, was spread throughout most length groups of sticklebacks examined (Figure 3) Uneven numbers in the distribution was probably due to the small sample size of fish examined in certain length groups (Table 5). Mean parasite burden apparantly increases with size of female sticklebacks and decreases with the size of male sticklebacks (Figure 4). Little significance, however, can be attached to the above distributions since the sample sizes in each length group are too small for reliability.

Figure 2

Seasonal change in the sex ratio of *Argulus foliaceus*

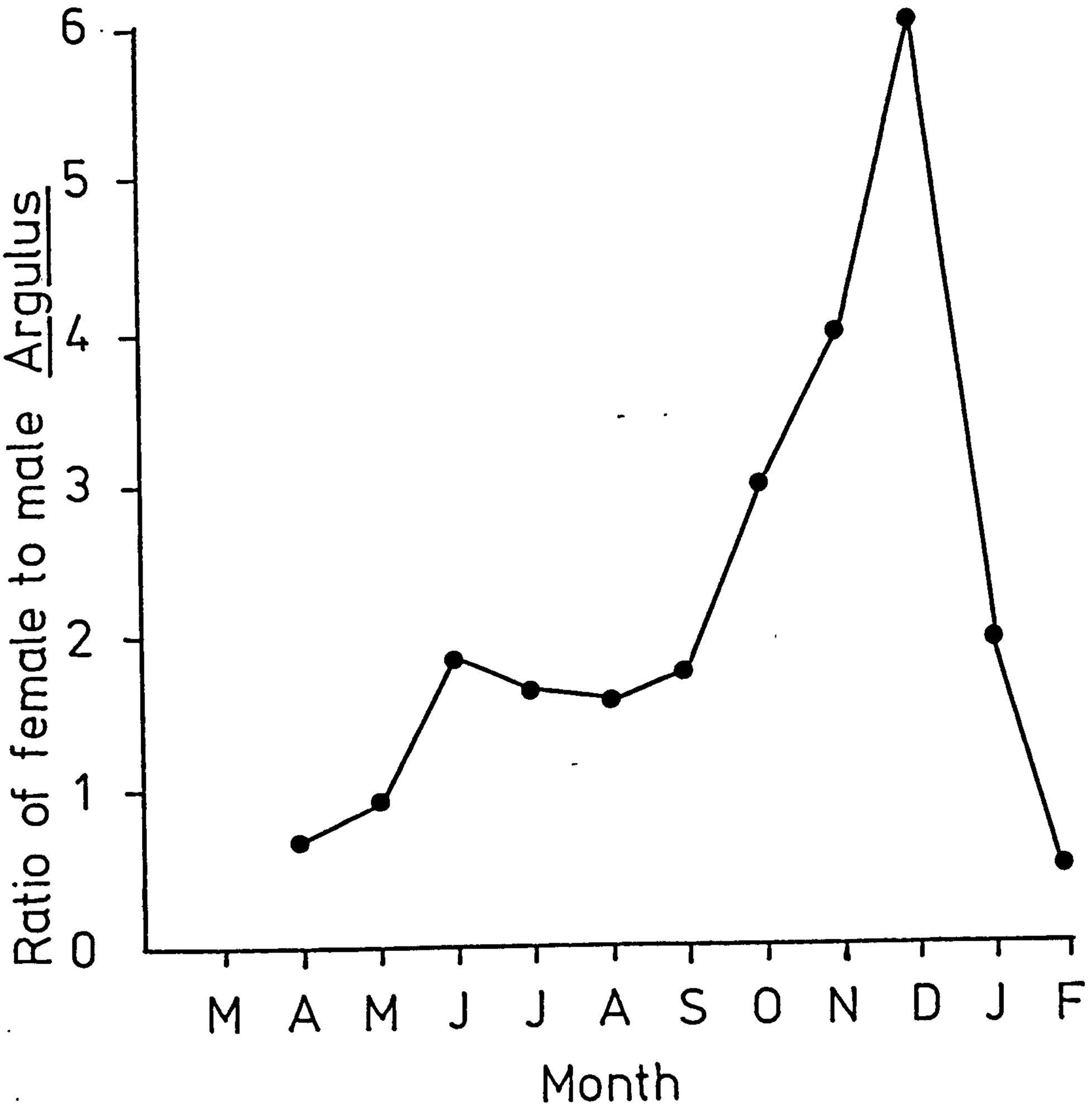

Ratio of female to male Argulus
0
1
2
3
4
5
6
M
A
M
J
J
A
S
O
N
D
J
F
Month

Figure 3

Change in the incidence of infection of sticklebacks by immature A.foliaceus in relation to the size of the fish

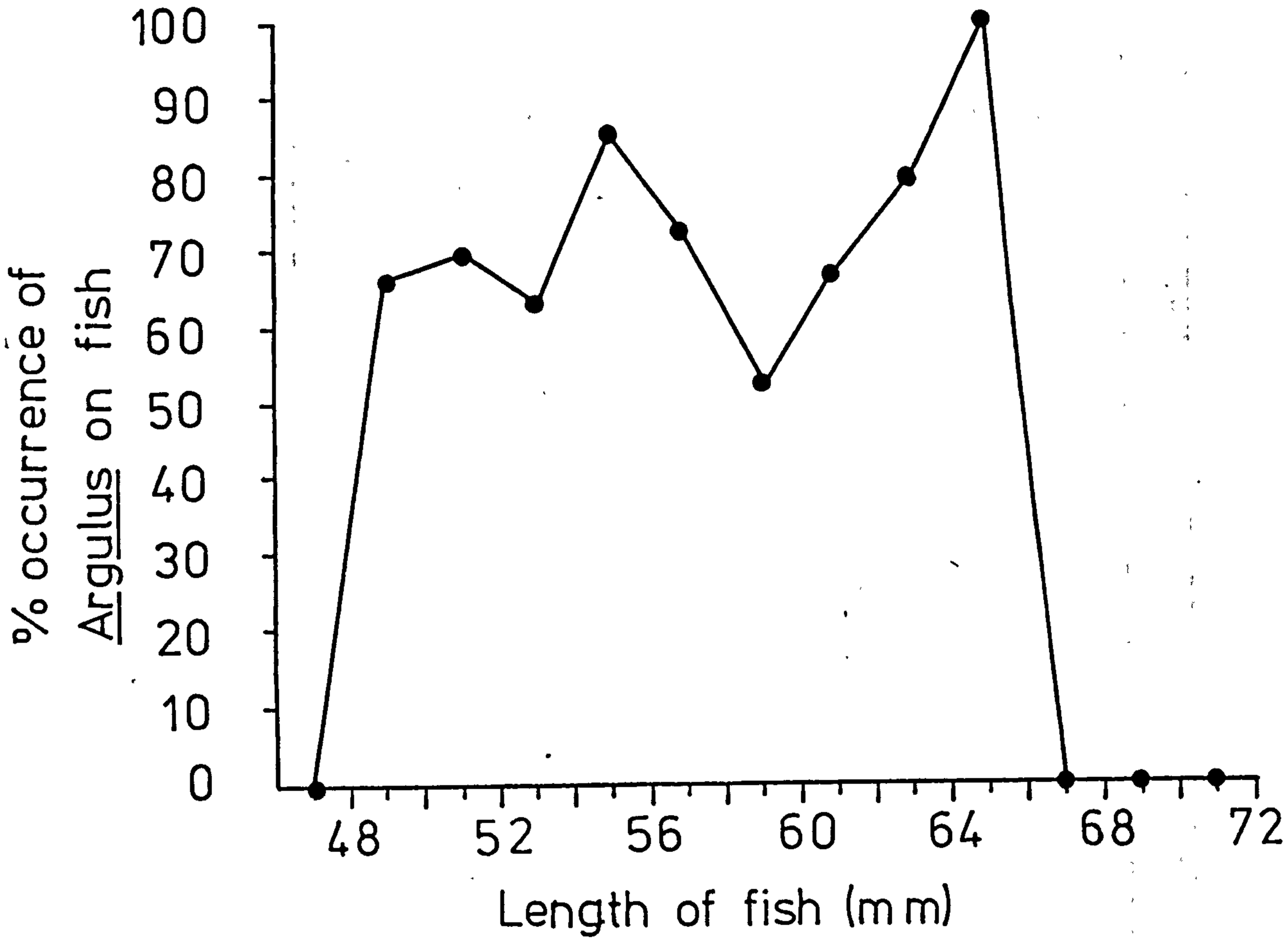

% occurrence of Argulus on fish
100
90
80
70
60
50
40
30
20
10
0
48
52
56
60
64
68
72
Length of fish (mm)

Table 5

Monthly sample of Gasterosteus aculeatus

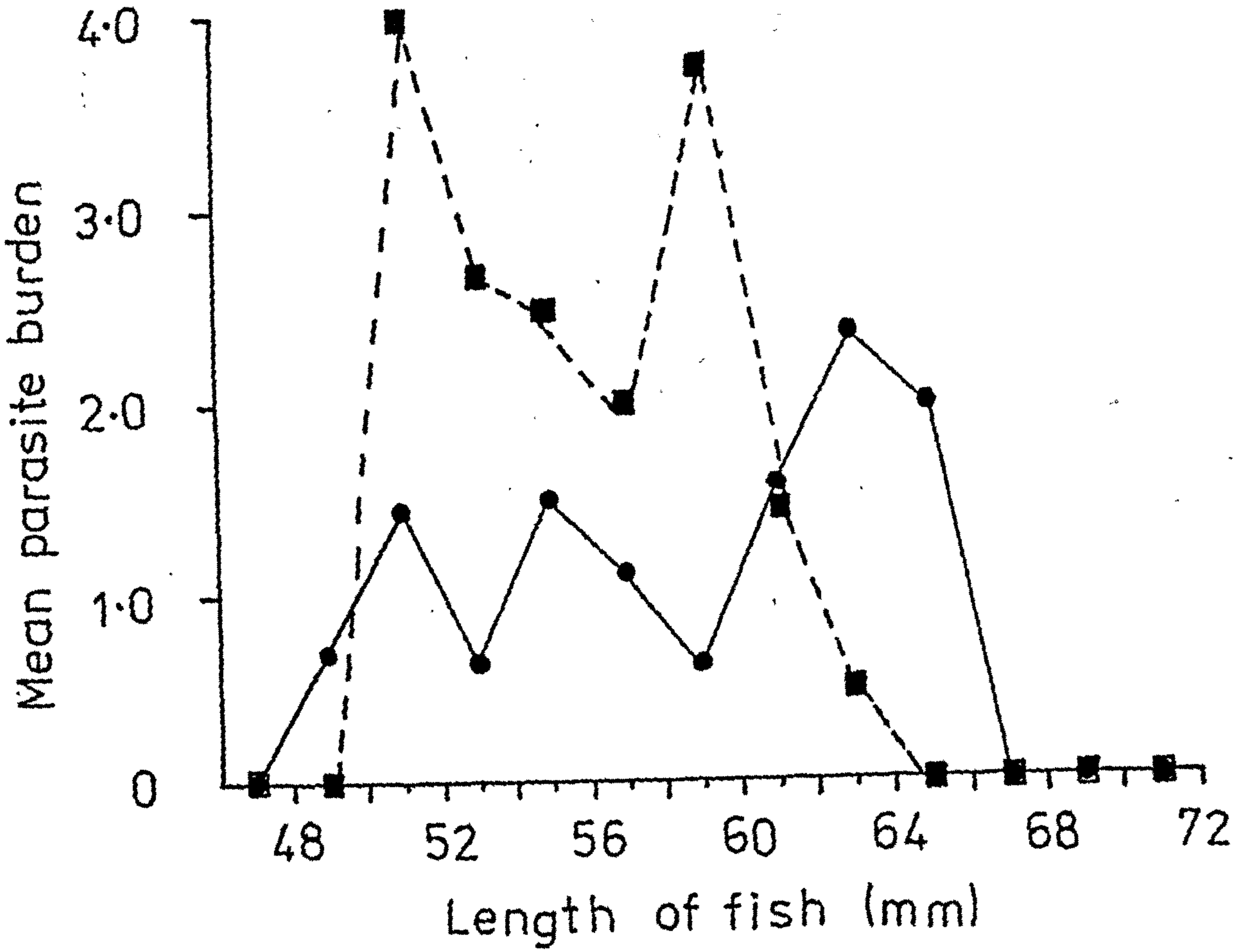
Length frequency (mm.)	Total number of fish	Female	Male
46 - 47.9	1	1	-
48 - 49.9	3	3	-
50 - 51.9	10	9	1
52 - 53.9	11	8	3
54 - 55.9	22.	14	8
56 - 57.9	15	11	4
58 - 59.9	17	13	4
60 - 61.9	9	7	2
62 - 63.9	10	8	2
64 - 65.9	2	2	-
66 - 67.9	0	-	-
68 - 69.9	1	1	-
70 - 71.9	1	1	-

Figure 4

Change in the intensity of infection of sticklebacks by immature A.foliaceus in relation to the sex and size of the fish

■ Male sticklebacks

● Female sticklebacks


Mean parasite burden
4·0
3·0
2·0
1·0
0
48
52
56
60
64
68
72
Length of fish (mm)

The frequency distribution of immature A.foliaceus on sticklebacks is shown in Figure 5 . The parameters and test statistics for the distribution are given in Table 6 . As the value of d is greater than 1.96, agreement with a Poisson series is rejected at the 95 % probability level, indicating that the parasite population was not randomly dispersed within the host population. A high value for d and also a variance to mean ratio of greater than one indicates an overdispersed distribution. While the negative binomial model is often used to describe such parasite distributions, it did not provide a good fit to the data.

(c) Site selection

The site distribution of immature A.foliaceus on field samples of G.aculeatus is shown in Table 7 . Analysis of possible between the distribution of A.foliaceus on the left or right sides of the body and fins, or between the total numbers present on the body or fins is given by χ^2 test. The results indicate that there is no significant difference in the site distribution of A.foliaceus on the left or right sides of the body and fins. The difference between the total numbers attached to the body and fins/spines is also not significant. The results therefore suggest that there are no preferential sites for the distribution of immature (approximately less than one week old) A.foliaceus on G.aculeatus.

2. Infection

(a) First attack

Results were treated by the use of probit analysis (Section 11.4). By a straightforward plot of percentage parasitism against time, a series of sigmoid curves were obtained for all the experimental densities of A.foliaceus used. Figure 6 indicates one such typical curve when five female A.foliaceus were exposed to one G.aculeatus per tank in 15 trials. The percentage parasitism at each ten minute interval thus represents

Figure 5

The frequency distribution of

immature A.foliaceus on G.aculeatus

from one monthly sample

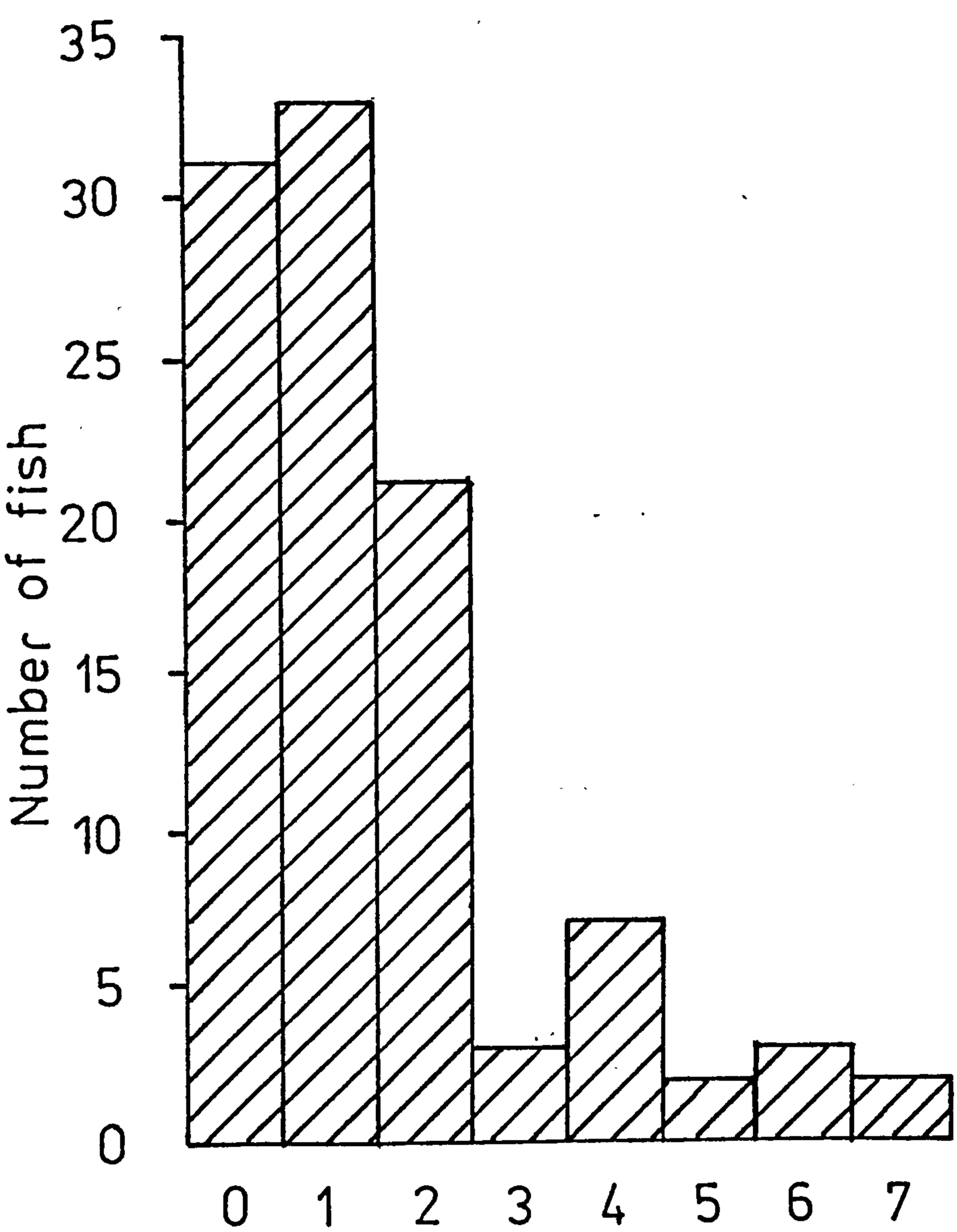

35
30
25
20
15
10
5
0
Number of fish
0 1 2 3 4 5 6 7
Number of parasites per fish

Table 6

Parameters and test statistics for the distribution of A.foliaceus on G.aculeatus

Number of fish	102
Number of A.foliaceus attached	154
Mean	1.5098
Variance	2.7672
Chi-square	185.11
d	5.0640
Variance/mean ratio	1.8328

Table 7

Site distribution of A.foliaceus on G.aculeatus (field results)

Site	Total	Position		Chi-square	Significance
		Left	Right		
Fins	76	37	39	0.0526	n/s
Caudal	23	8	15	2.1304	n/s
Anal	12	6	6	0.0000	n/s
Dorsal	23	12	11	0.0435	n/s
Pectoral	18	11	7	0.8889	n/s
Body	53	24	29	0.4717	n/s
Anterior	20	9	11	0.2000	n/s
Middle	19	9	10	0.0526	n/s
Posterior	14	6	8	0.2857	n/s
Ventral	11	-	-	-	-
Dorsal	4	-	-	-	-
		Ventral	Dorsal		
Spines	10	8	2	3.6000	n/s
		Body	Fins + spines		
Total attached	154	68	86	2.1039	n/s

Null Hypothesis is that there should be no significant difference between the attachment of A.foliaceus on either the right or left sides of G.aculeatus.

the percentage number out of 15 G.aculeatus which had been attacked for the first time at that particular density of female A.foliaceus. The sigmoid curve is transformed by probit analysis into a straight line from which it is possible to estimate the mean time (ED 50) for 50 percent of the fish population to be attacked for the first time at that particular density (Figure 7). All results were treated in a similar manner and a detailed example is given in Appendix 1. Equations for the regression lines produced by probit analysis and the resulting ED 50's both for female and male A.foliaceus when exposed to one G.aculeatus are given in Table 8. When the ED 50's are plotted against parasite density, a negative exponential curve of the form ED 50 (p) = ED 50 (o) exp ($-\alpha p$) is obtained for both female and male A.foliaceus (Figures 8 and 9). With both female and male A.foliaceus, increases in parasite density decreases the time to first attack, but it does so at a constant exponential rate. Further analysis of these results is considered in the discussion (page 43).

Equations for the regression lines and the resulting ED 50's when 20 female A.foliaceus are exposed to increasing densities of G.aculeatus are given in Table 9 . It can be seen that increasing host density also decreases the time to first attack and it does so at a constant exponential rate (Figure 10). Further analysis of these results is considered in the discussion (page 43).

Results for 30 male A.foliaceus exposed to different host species are given in Table 10. Comparison by χ^2 of the ED 50's obtained when 30 male A.foliaceus are exposed to different host species indicates no significant difference between C.carassius, G.aculeatus and P.phoxinus. There is, however, a significant difference between these three species and N.barbatulus ($P > 0.05$).

(b) Infection pattern

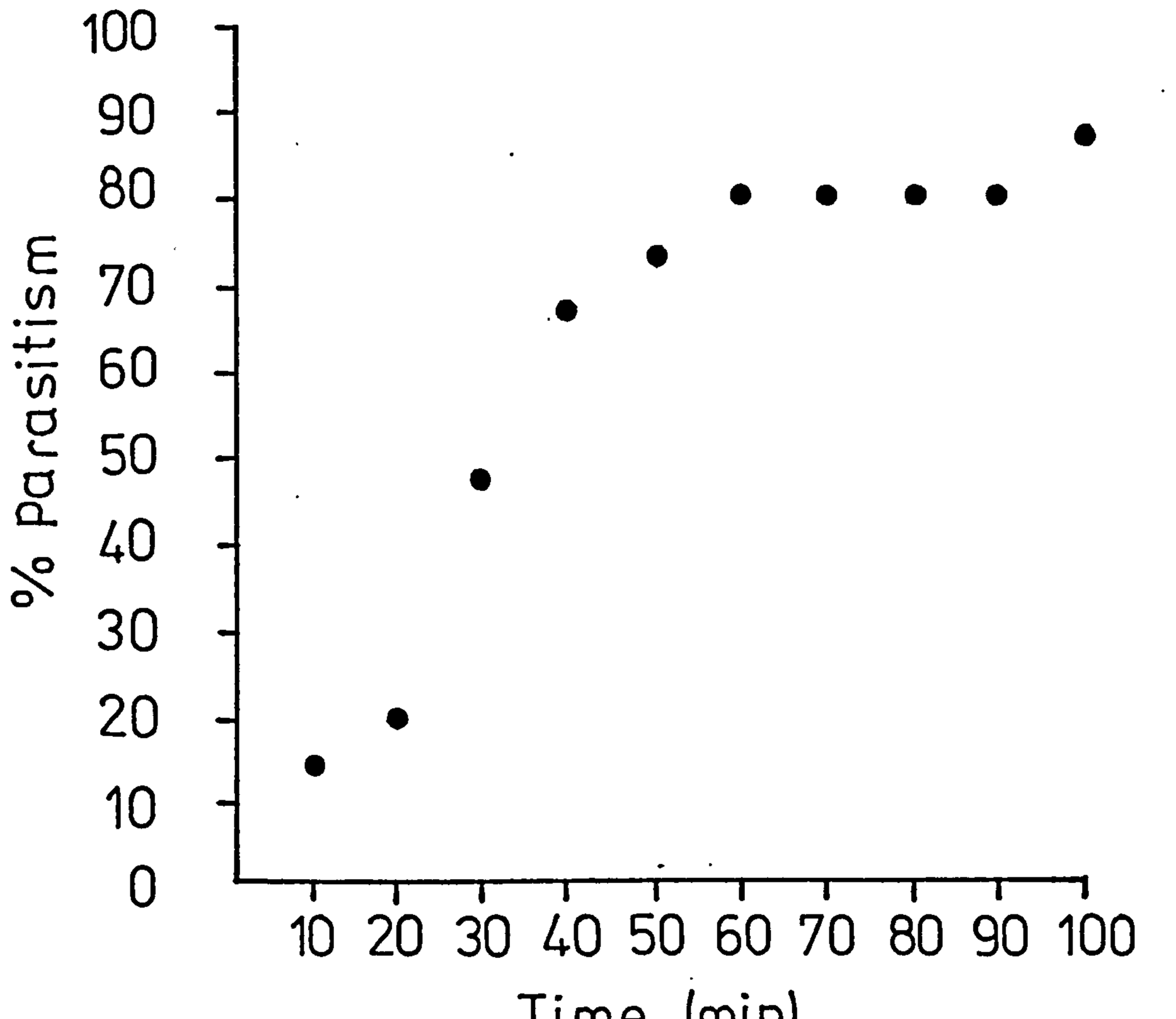
The infection patterns observed when different densities of female

Figure 6

Figure 7

Example at one particular density of female A.foliaceus,showing the percentage parasitism amongst the stickleback population which had been attacked for the first time over a given time period

Transformation of the above graph by the use of probit analysis showing the regression line obtained


% Parasitism
100
90
80
70
60
50
40
30
20
10
0
10 20 30 40 50 60 70 80 90 100
Time (min)

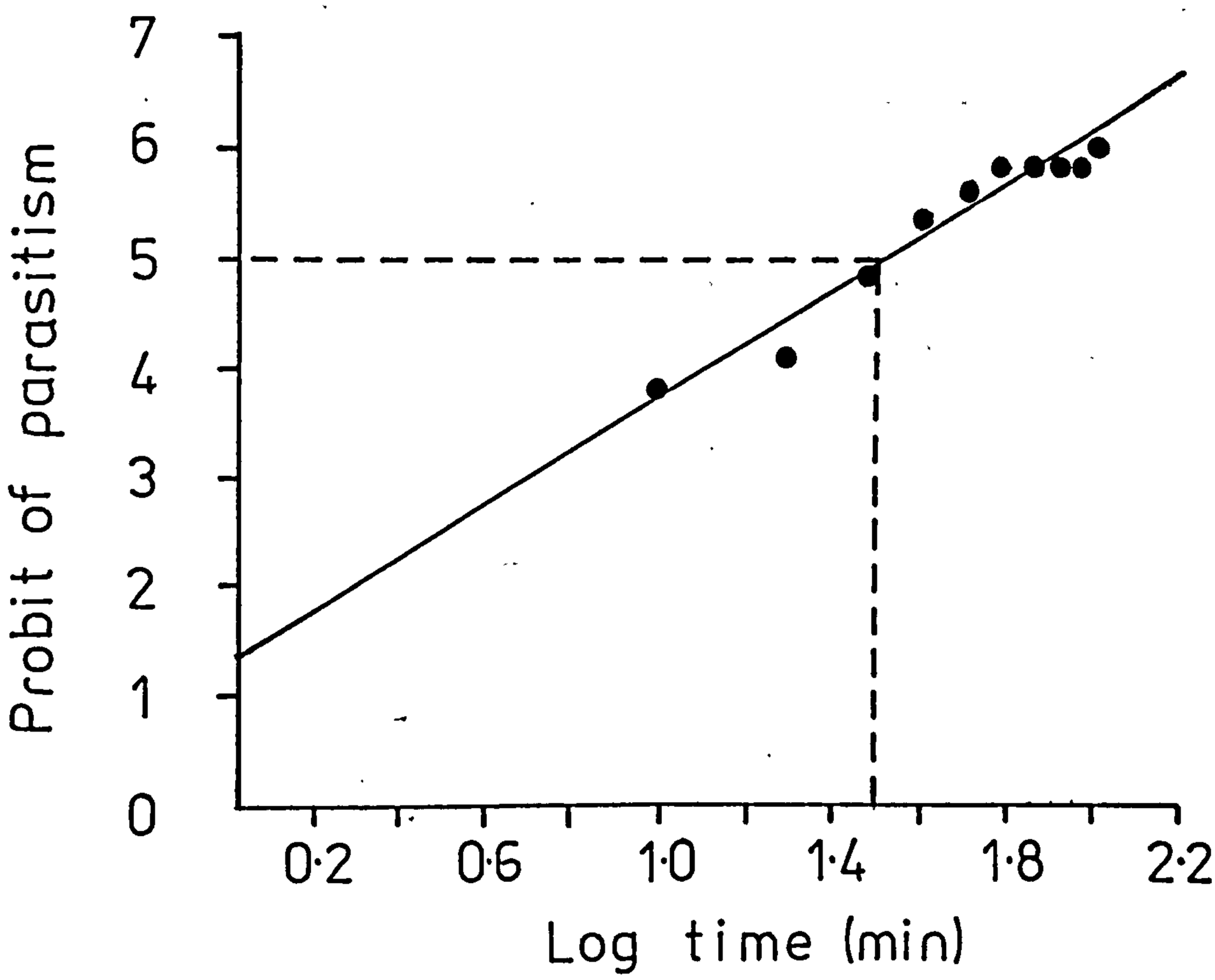

Probit of parasitism
7
6
5
4
3
2
1
0
0·2 0·6 1·0 1·4 1·8 2·2
Log time (min)

Table 8

First attack by A.foliaceus onto one G.aculeatus

Density of female A.foliaceus	Linear equation	ED 50
5	Y = 1.413 + 2.380x	32.359 min.
10	Y = 2.094 + 2.012x	27.843 min.
15	Y = 3.360 + 1.350x	16.410 min.
20	Y = 2.943 + 1.808x	13.731 min.
25	Y = 3.797 + 1.239x	9.360 min.
30	Y = 3.952 + 1.375x	5.790 min.
35	Y = 4.127 + 1.543x	3.680 min.
40	Y = 4.022 + 1.585x	4.139 min.
45	Y = 4.320 + 1.899x	2.280 min.
50	Y = 2.162 + 1.956x	28.277 sec.
Density of male A.foliaceus	**Linear equation**	**ED 50**
5	Y = 1.900 + 2.122x	28.897 min.
10	Y = 2.060 + 2.326x	18.361 min.
15	Y = 3.136 + 1.722x	12.088 min.
20	Y = 3.734 + 1.451x	7.453 min.
25	Y = 3.692 + 1.307x	10.018 min.
30	Y = 4.648 + 0.802x	2.748 min.

Figure 8

The effect of increasing parasite density

on the first attack of female Argulus foliaceus

Solid line represents the best fit exponential model of the form:-

$$N(t) = x \exp(-yt)$$

where $x = 62{\cdot}115$

$y = 0{\cdot}0811$

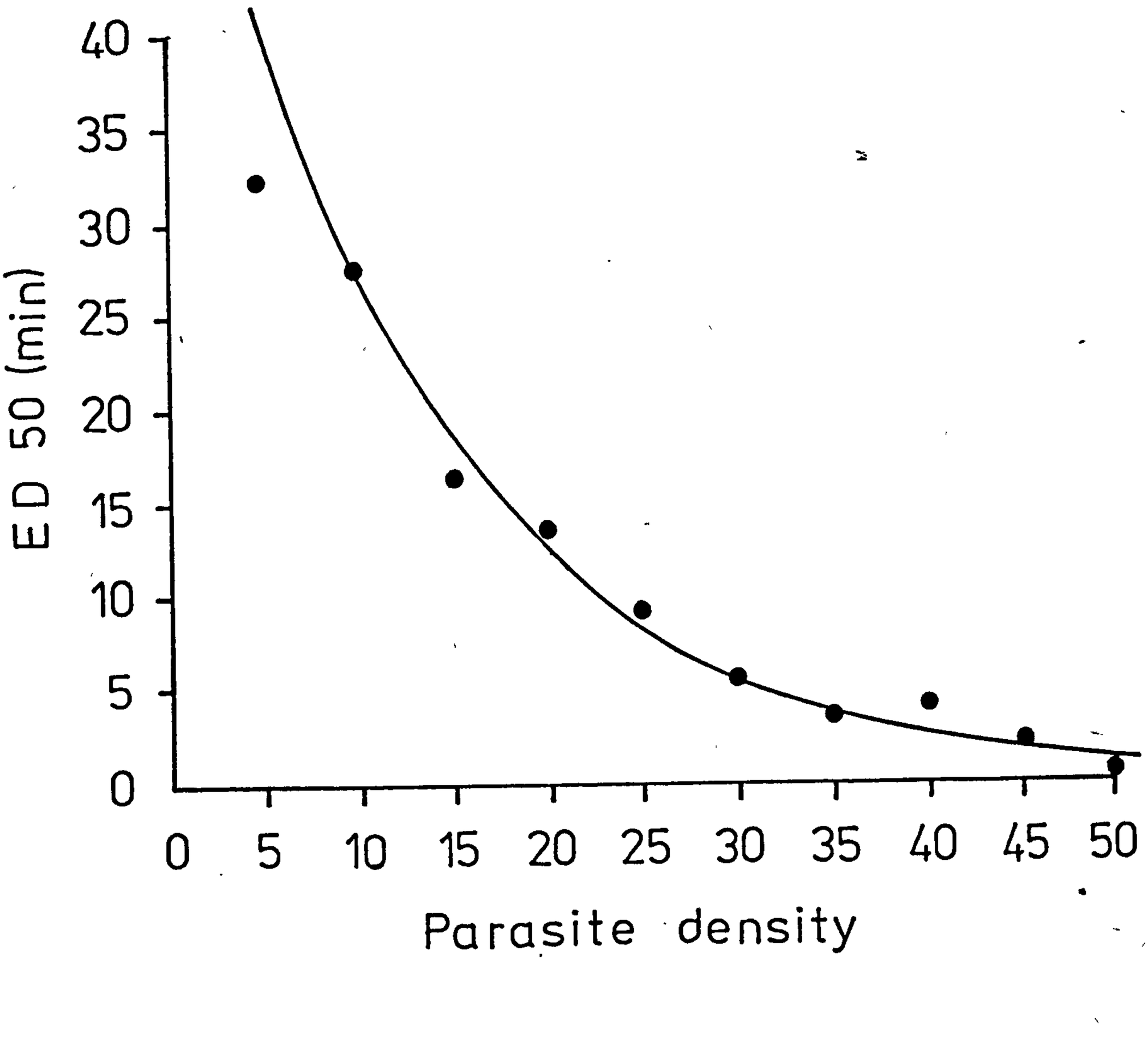

ED 50 (min)
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
45
50
Parasite density

Figure 9

The effect of increasing parasite density on the first attack of male Argulus foliaceus

Solid line represents the best fit exponential model of the form:-

$$N(t) = x \exp(-yt)$$

where $x = 42.7268$

$$y = 0.0804$$

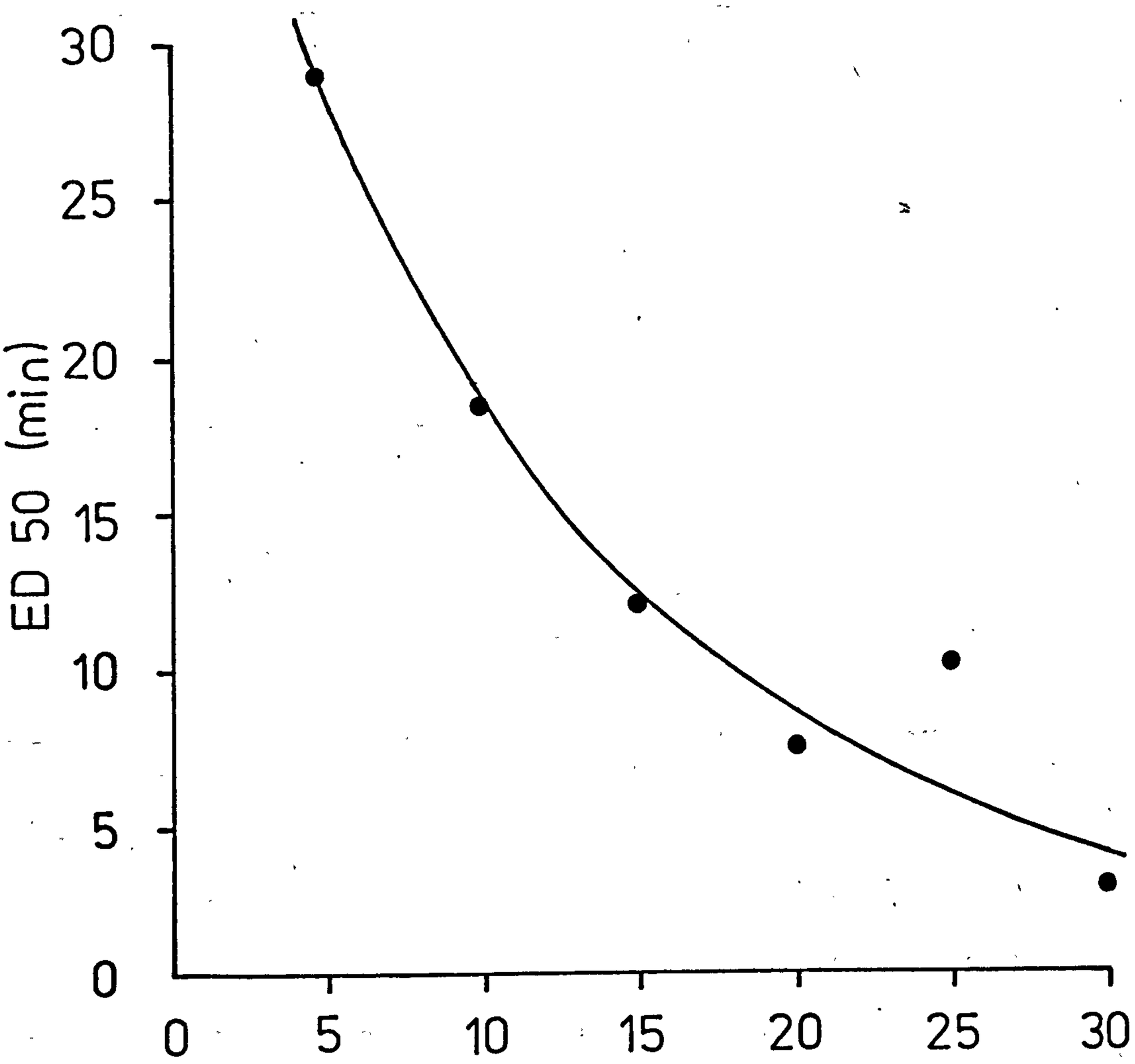

ED 50 (mic)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
Parasite density

Table 9

First attack by 20 female A.foliaceus onto different densities of G.aculeatus

Density of G.aculeatus	Linear equation	ED 50
1	Y = 2.943 + 1.808x	13.731 min.
2	Y = 3.923 + 2.767x	2.986 min.
3	Y = 4.000 + 1.480x	2.543 min.
4	Y = 4.233 + 2.673x	1.935 min.
5	Y = 2.325 + 1.680x	39.162 sec.

Table 10

First attack by 30 male A.foliaceus exposed to different species

Host species	Linear equation	ED 50
Carassius carassius	Y = 4.586 + 1.003x	2.589 min.
Gasterosteus aculeatus	Y = 4.648 + 0.802x	2.748 min.
Phoxinus phoxinus	Y = 4.440 + 0.898x	4.205 min.
Noemacheilus barbatulus	Y = 2.512 + 1.560x	39.305 min.

Figure IO

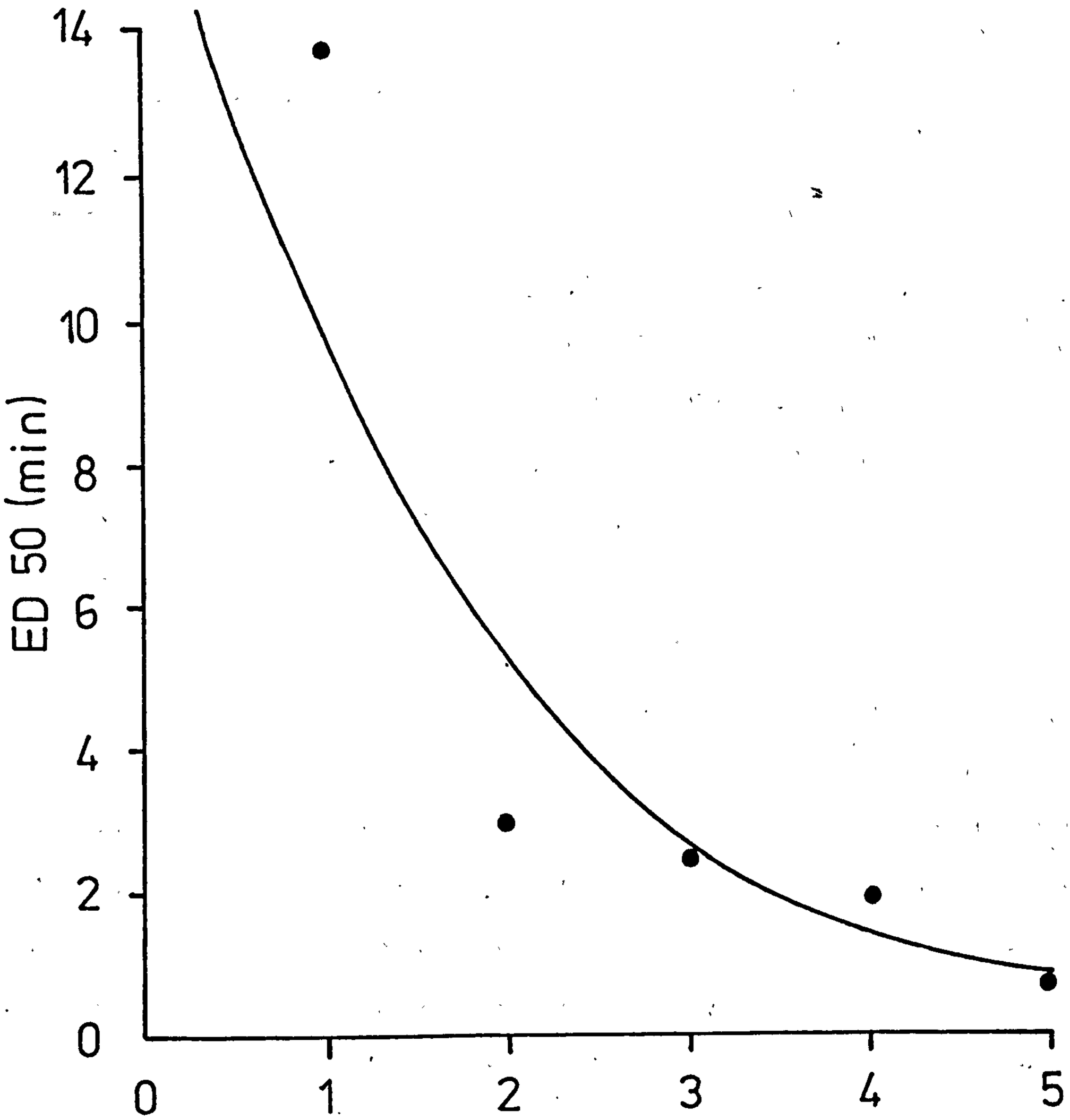
The effect of increasing fish host density on the first attack of female Argulus foliaceus

Solid line represents the best fit exponential model of the form :-

$$N(t) = x \exp(-yt)$$

where $x = 18.8018$

$y = 0.6526$


14
12
10
8
6
4
2
0
ED 50 (min)
0
1
2
3
4
5
Host density

and male A.foliaceus are exposed to one G.aculeatus over a 12 hour period are given in Figures 11 and 12 . Each point is based on a mean number of five trials and the results are expressed as the mean number of A.foliaceus at each density which had infected the fish at each half hour interval. The infection pattern when 20 male A.foliaceus are exposed to different host species is similarly expressed in Figure 13.

Similar patterns are observed at all densities of both female and male parasites. In all cases, infection stabilises and there is always a residual free living A.foliaceus population which do not infect the fish. The mathematical model used to interpret these results has been discussed previously (Section 11.4. page 25) and will be considered later (page 47 and Appendix 2).

The infection of male A.foliaceus onto four different fish species shows a similar pattern to infection onto sticklebacks. However, the final numbers establishing on these species shows a distinct difference. Analysis of these results is considered later (page 50).

(c) Attack rate

The relationship between the length of time taken between successive attacks when a fixed density of female A.foliaceus are exposed to a single host is shown in Figure 14. Each mean is based on 15 trials. With increasing attacks, the length of time taken between attacks increases exponentially. The relationship between the effect of first attack on second attack, second attack on third attack, third attack on fourth attack and fourth attack on fifth attack by correlation analysis (in terms of the mean length of time taken for each attack) indicates an increasing correlation, where $r = 0.7347, 0.7956, 0.8037$ and 0.8964 respectively. This suggests that successive attacks are increasingly affected by the previous attack.

Figure 11

The relationship between the mean number of successful infections per host by different densities of female Argulus foliaceus and the duration of exposure to infection

Parasite density

5 ■

10 ○

20 □

50 ●

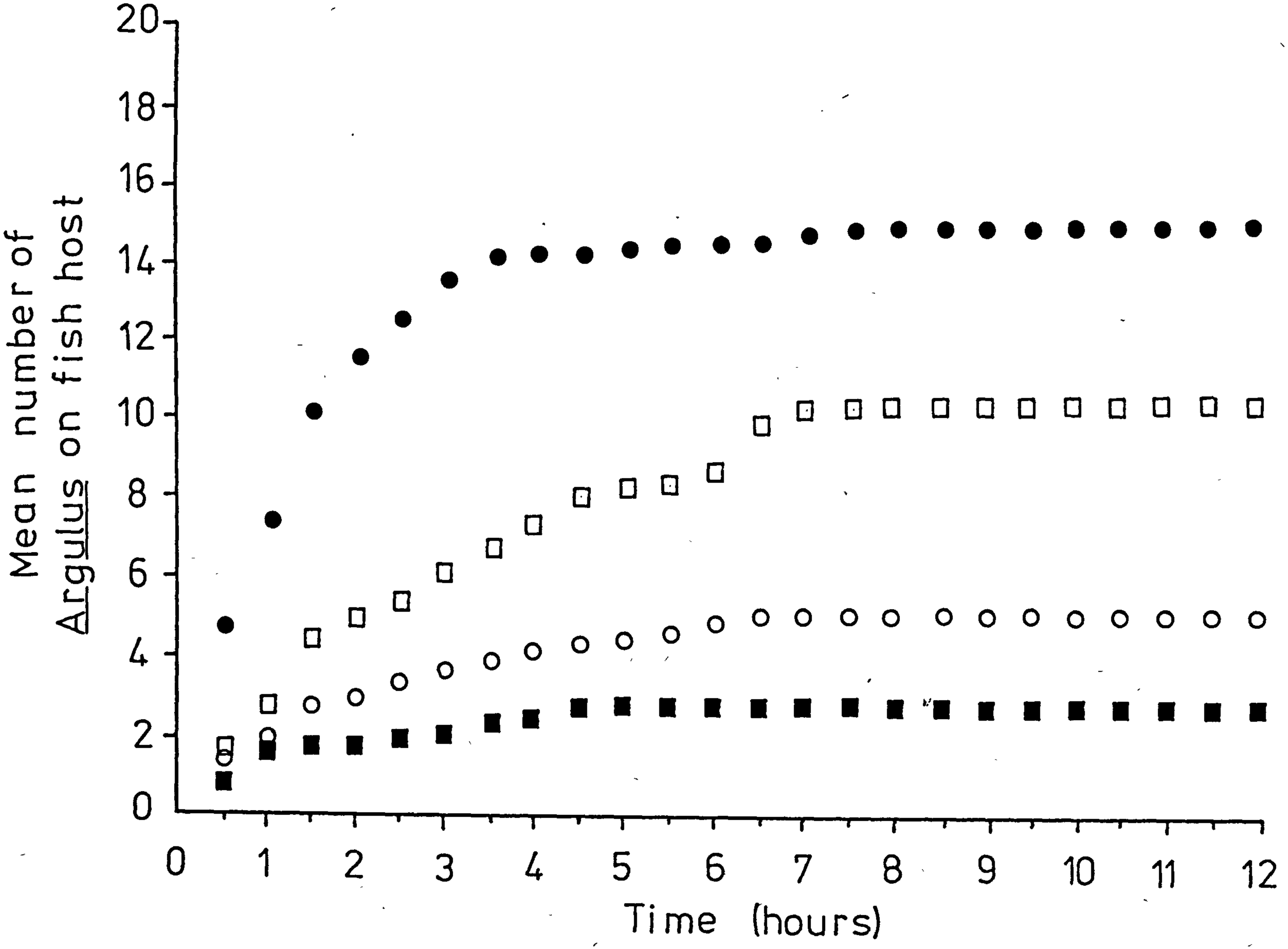

Mean number of
Argulus on fish host
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (hours)

Figure 12

The relationship between the mean number of successful infections per host by different densities of male Argulus foliaceus and the duration of exposure to infection

Parasite density

5 ■

10 ○

20 □

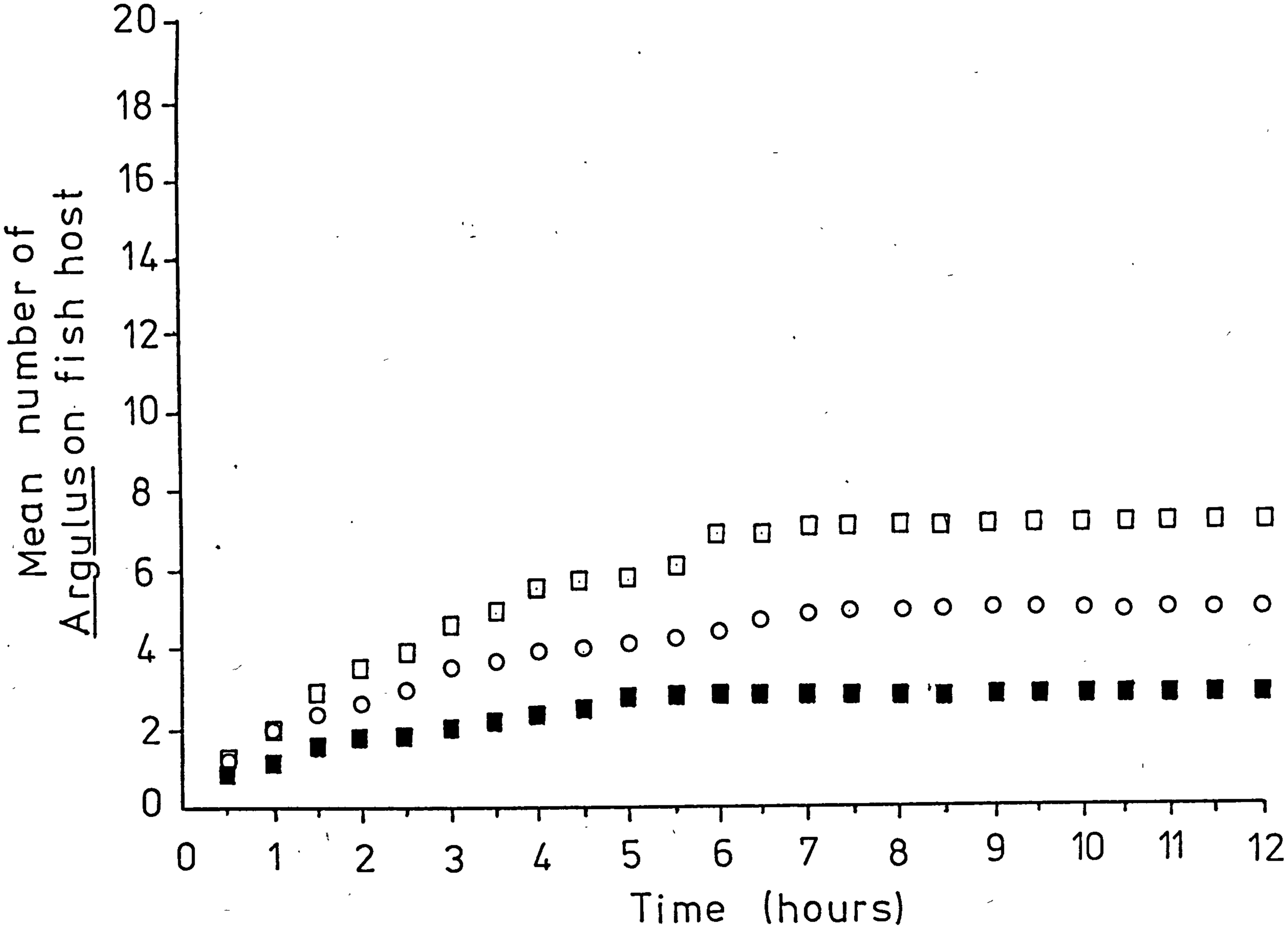

Mean number of
Argulus on fish host
0
2
4
6
8
10
12
14
16
18
20
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (hours)

Figure 13

The relationship between the mean number of successful infections per host by a fixed density (20) of male Argulus foliaceus onto different host species and the duration of exposure to infection

Fish species

Carassius carassius ●

Phoxinus phoxinus □

Gasterosteus aculeatus ○

Noemacheilus barbatulus ■

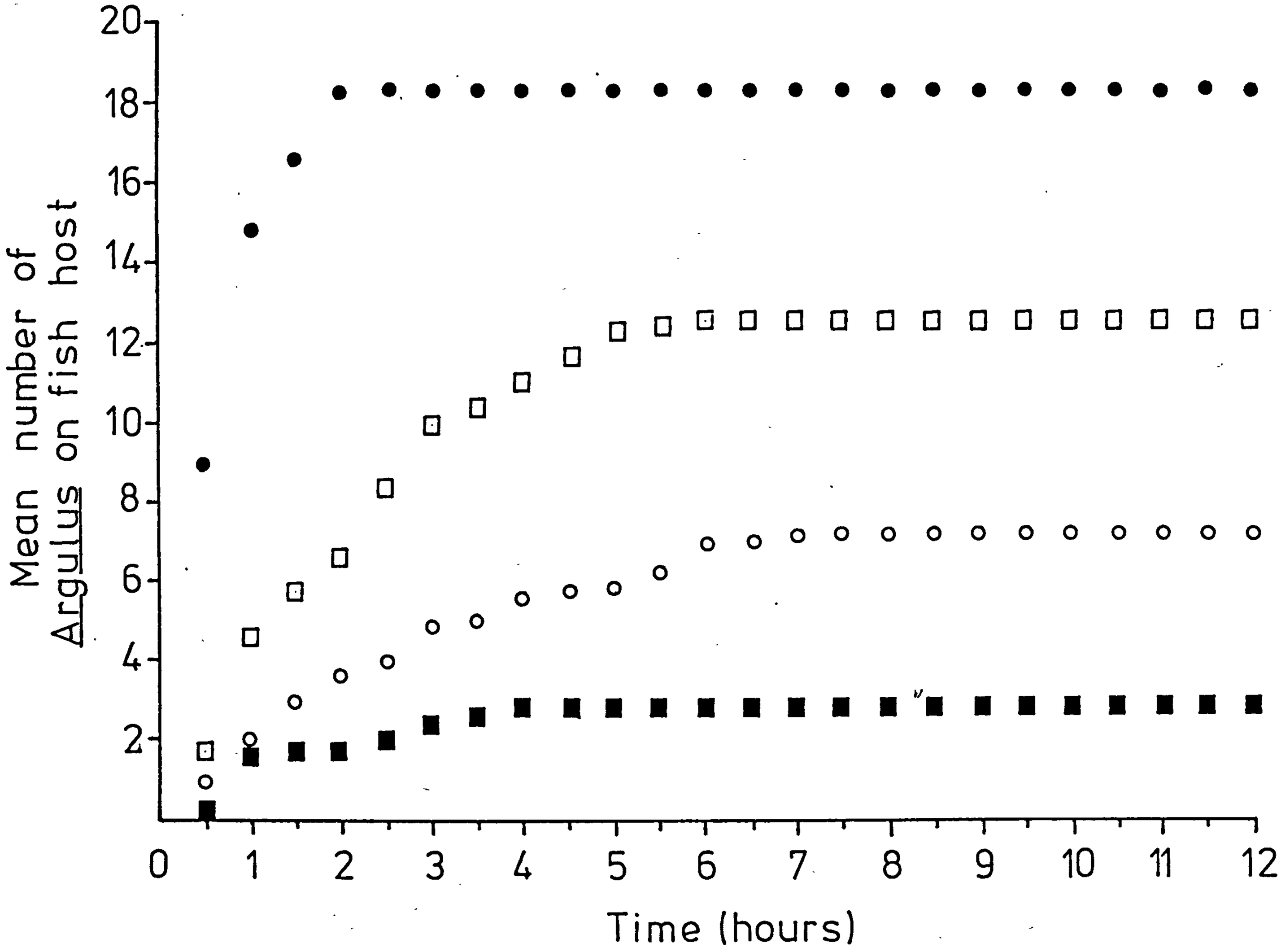
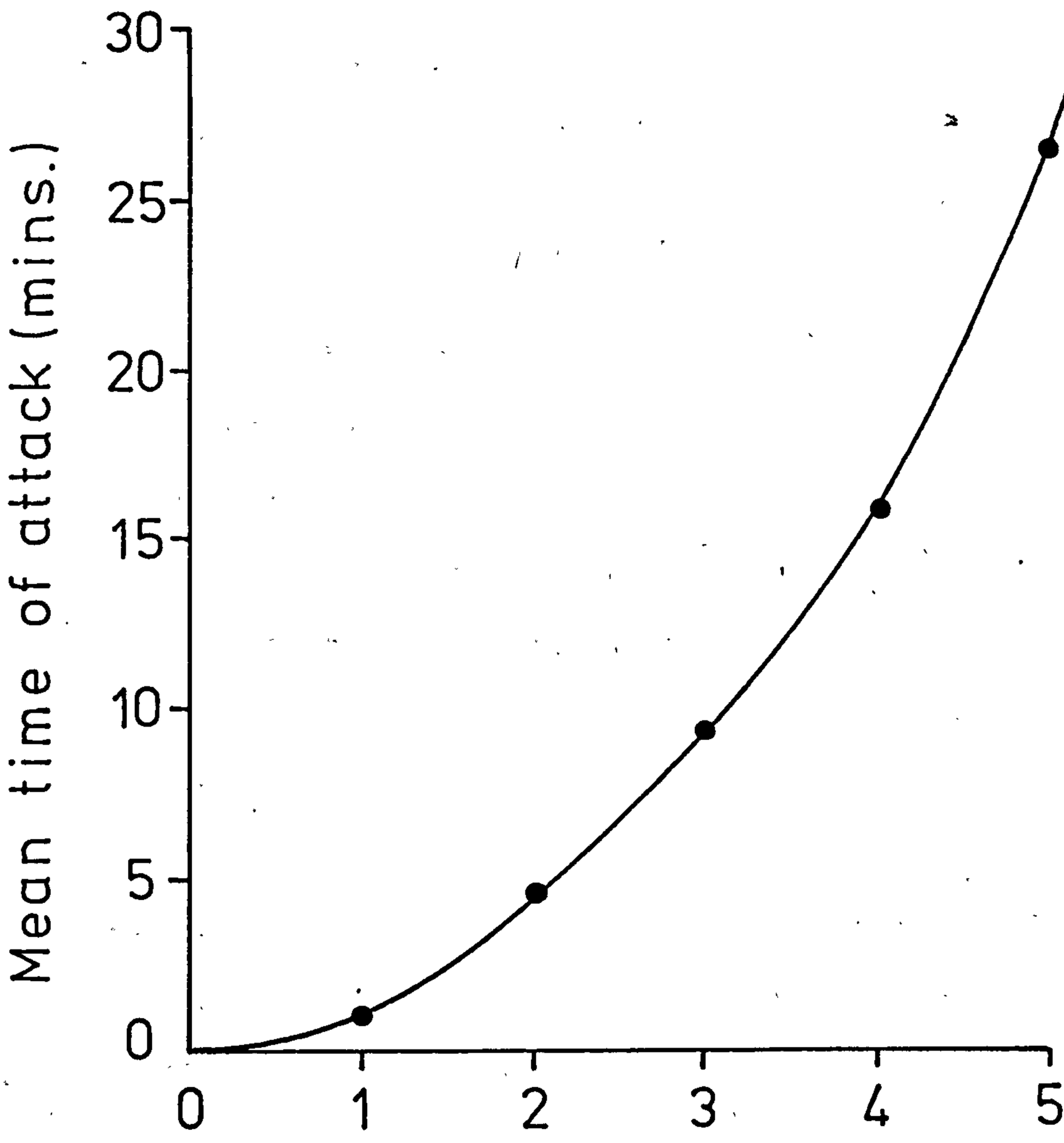

Mean number of
Argulus on fish host
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (hours)

Figure 14

The relationship between the length of time taken between successive attacks and the number of attacks

Exponential line fitted by eye.


Mean time of attack (mins.)
30
25
20
15
10
5
0
0
1
2
3
4
5
Number of attacks

(d) Effect of parasite and host density on infection

This series of experiments investigated infection over a short exposure period (10 minutes) as opposed to the longer exposure period (12 hours) previously mentioned (Section III.b.).

The relationship between the mean numbers of adult and larval A.foliaceus infecting one G.aculeatus over a 10 minute exposure period are given in Figures 15 and 16. Each mean is based on 20 trials and 95 % confidence limits are shown. With larvae, the mean number of infections increases approximately linearly with increasing parasite density, whereas with adults, the relationship tends towards a plateau. The mathematical model used to interpret these results has been described previously (Section II. 4. page 25) and further discussion is dealt with later (page 47)

In both larvae and adults, the variance in the number of parasite infections increases at a faster rate than the mean with increasing parasite density. The relationship between the variance to mean ratios and parasite density are shown in Figure 17 for adults and Figure 18 for larvae. The frequency distributions change in relation to the parasite density. At low densities, underdispersed distributions are apparant (variance less than mean), at intermediate levels, random distributions (variance equals mean)and at high parasite densities, overdispersed patterns are observed (variance greater than mean).

The mean numbers of adult and larval A.foliaceus attaching to increasing numbers of G.aculeatus after 10 minutes are given in Tables 11 and 12 . The relationship between mean numbers attached, host and parasite density for adults is given in Figure 19 . Since larval experiments were carried out in different volumes of water, no comparative figure is given. The mathematical model used to analyse these results has been described previously (Section II.4. page 26).and further discussion is dealt with later (page 50).

Figure 15

The relationship between the mean number of successful infections per host and the parasite (adult) density when exposed to one fish host

● = Observed means. Vertical bars represent 95% confidence limits of the means.

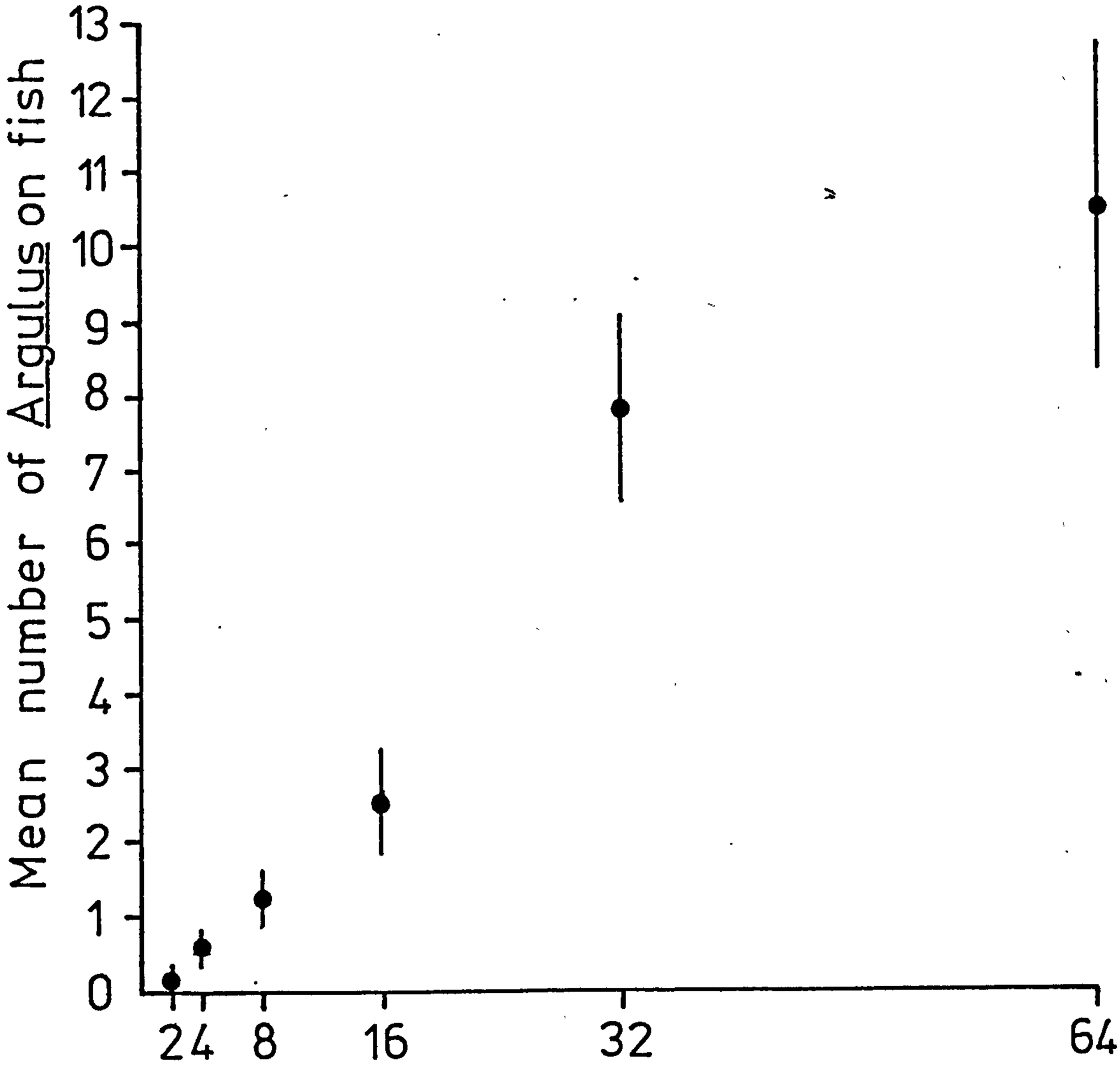

Mean number of Argulus on fish
0
1
2
3
4
5
6
7
8
9
10
11
12
13
24
8
16
32
64
Parasite density

Figure 16

The relationship between the mean number of successful infections per host and the parasite (larval) density when exposed to one fish host

● = Observed means. Vertical bars represent 95% confidence limits of the means.

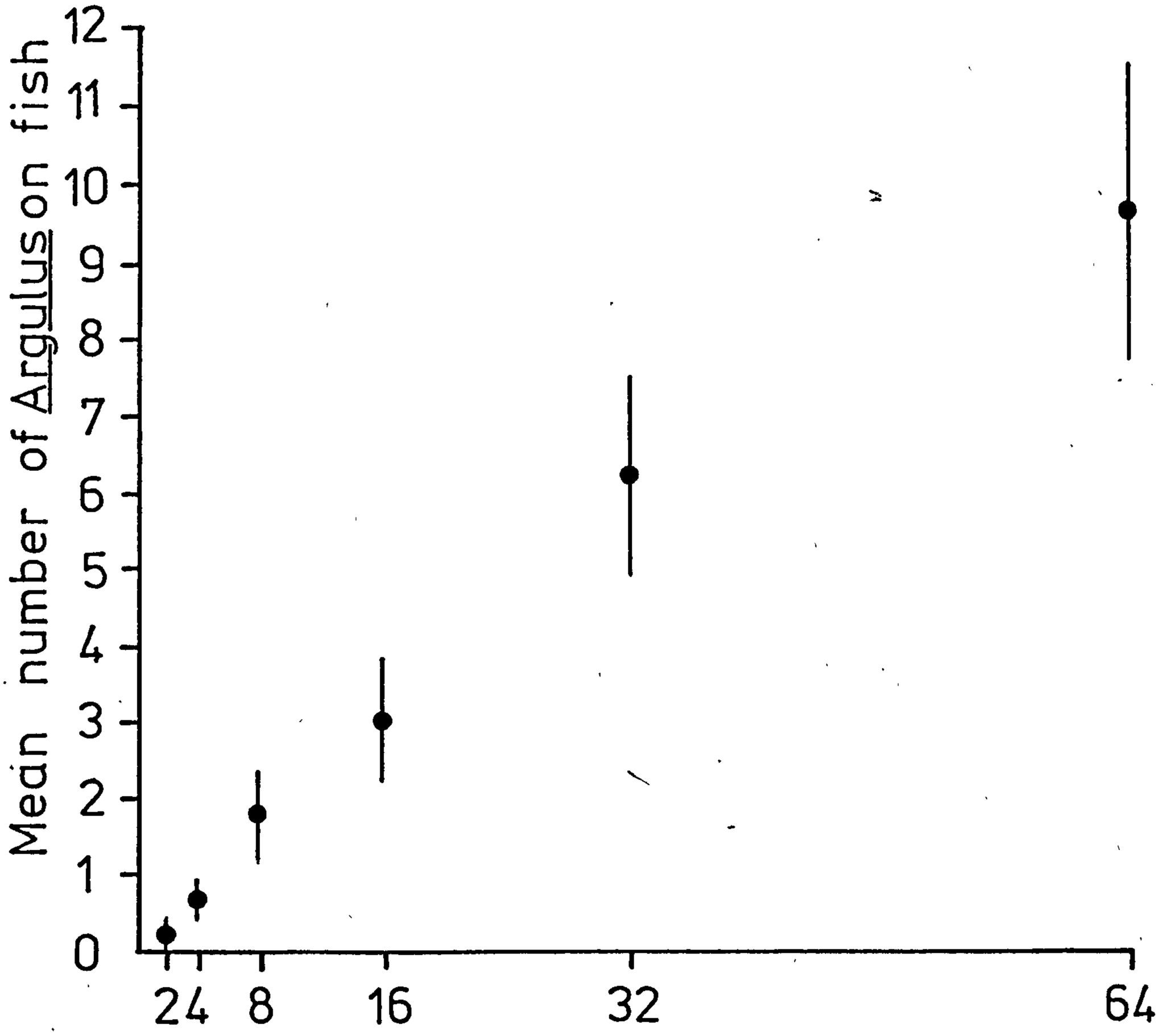

Mean number of Argulus on fish
0
1
2
3
4
5
6
7
8
9
10
11
12
2
4
8
16
32
64
Parasite density

Figure 17

The relationship between the variance to mean ratios of the number of successful infections per host and the parasite (adult) density when exposed to one host

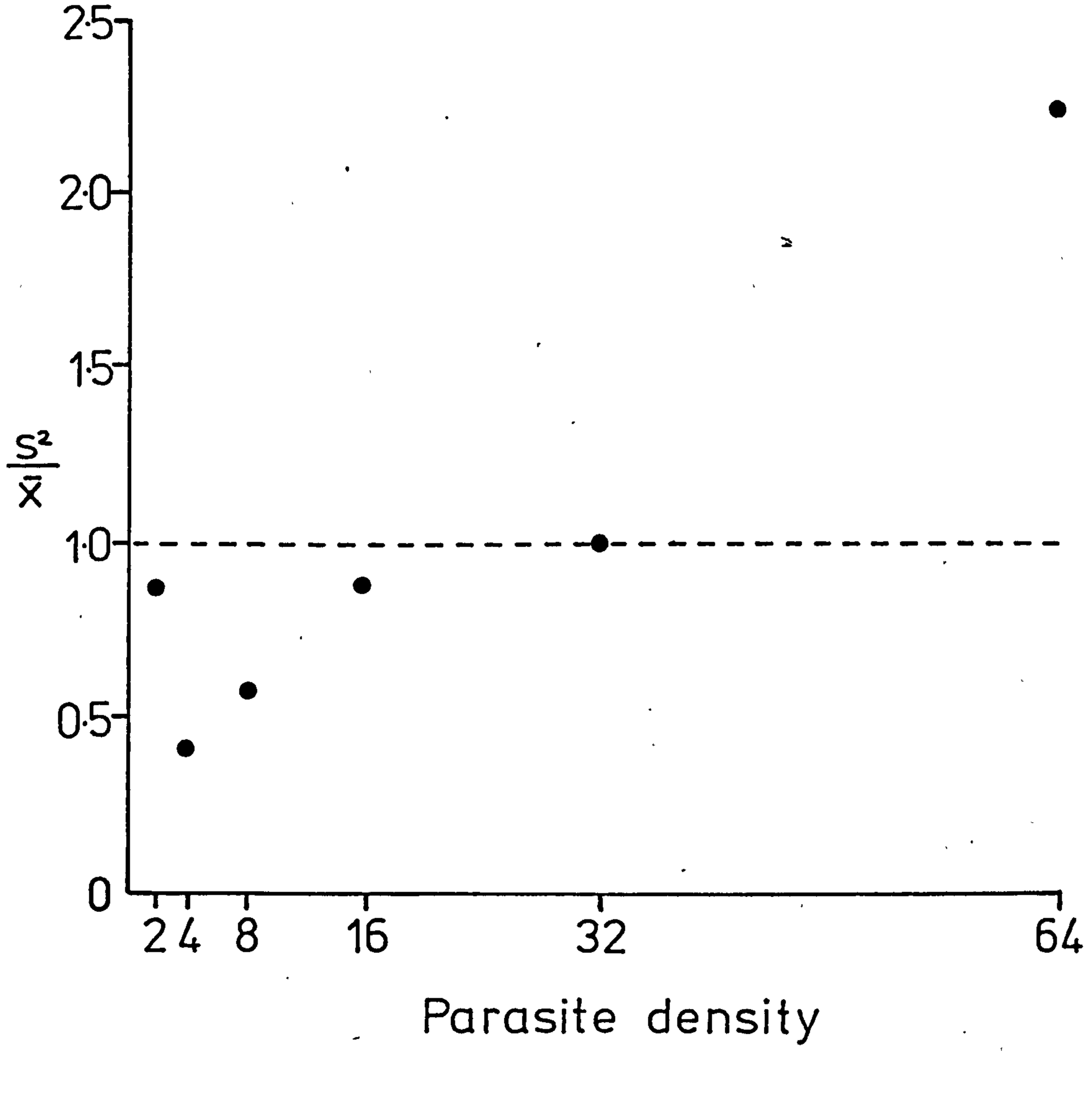

2·5
2·0
1·5
1·0
0·5
0
$\frac{S^2}{\bar{x}}$
2
4
8
16
32
64
Parasite density

Figure 18

The relationship between the variance to mean ratios of the number of successful infections per host and the parasite (larval) density when exposed to one host

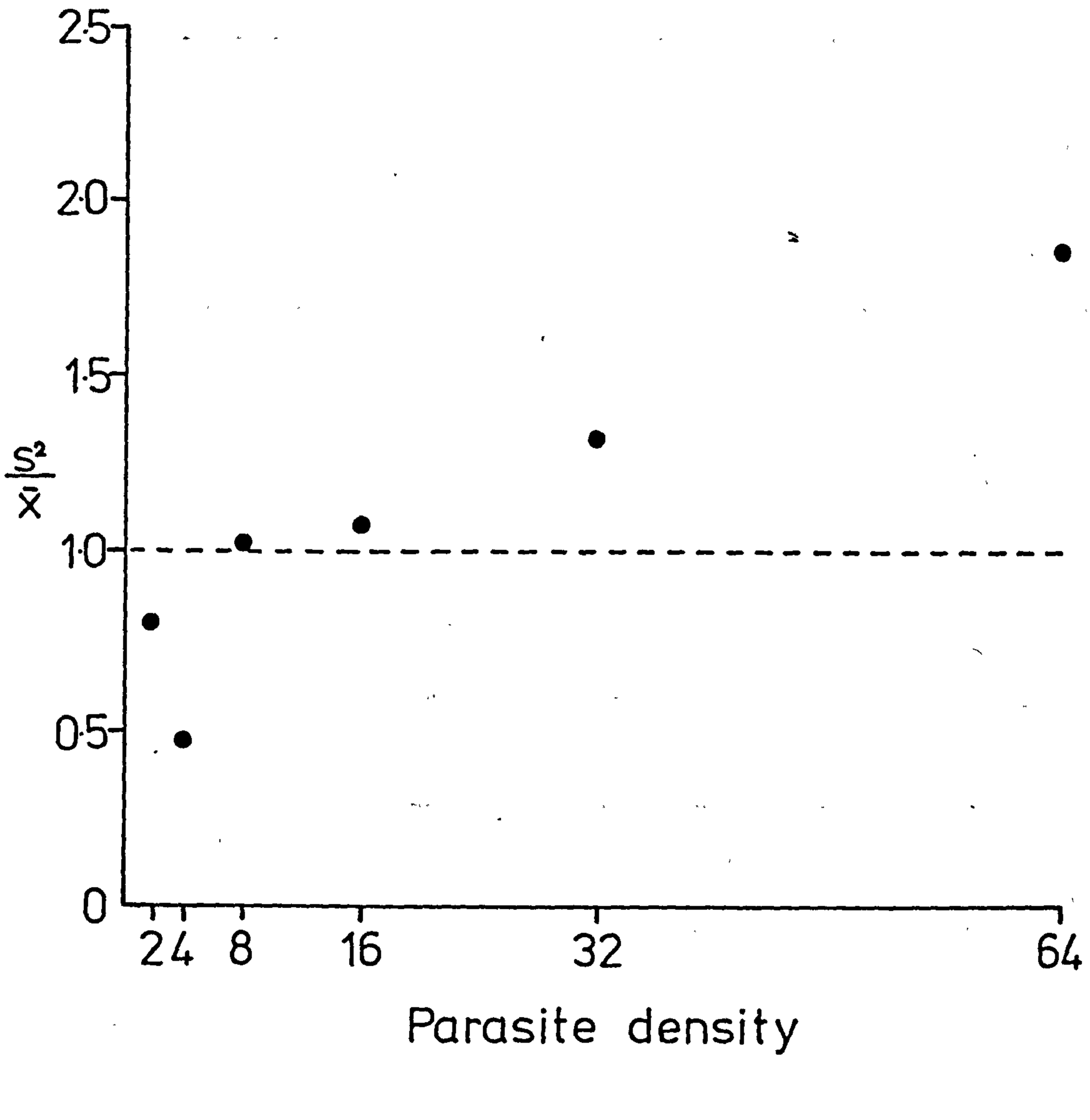

2·5
2·0
1·5
1·0
0·5
0
$\frac{S^2}{\bar{X}}$
2 4 8 16 32 64
Parasite density

Table 11

Effect of increasing host density on adult A.foliaceus infection (figures represent the mean numbers of parasites attached)

Density of hosts	Density of parasites					
	2	4	8	16	32	64
1	0.15	0.60	1.25	2.55	7.85	10.50
2	1.30	1.95	2.40	3.95	7.95	13.70
4	-	2.35	3.10	5.60	9.95	18.10
8	-	-	3.55	4.25	17.15	17.20

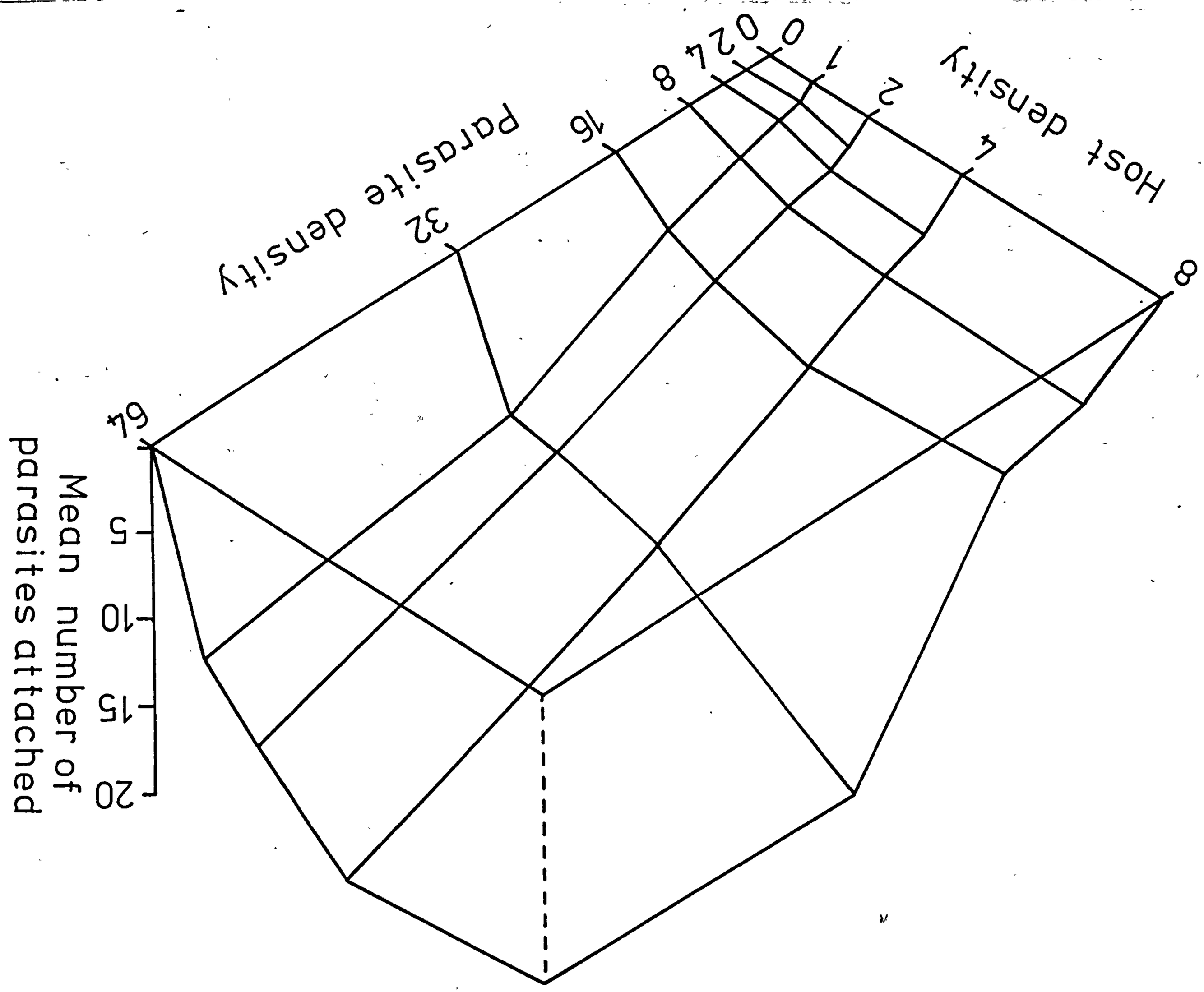
Table 12

Effect of increasing host density on larval A.foliaceus infection (figures represent the mean numbers of parasites attached)

Density of hosts	Density of parasites					
	2	4	8	16	32	64
1	0.25	0.70	1.80	3.05	6.25	9.65
2	0.30	1.05	1.70	2.05	2.35	4.20
4	-	0.90	1.05	1.20	2.15	5.30
8	-	-	1.70	2.45	3.25	4.15

Figure 19

The relationship between the mean numbers attached, host and parasite density for A.foliaceus adults


Mean number of parasites attached
5
10
15
20
64
32
16
8
4
2
1
0.8
0.4
0.2
0
Parasite density
Host density

(e) Distribution and site selection

(i) Dispersion pattern

The observed frequency distribution of A.foliaceus larvae on sticklebacks is shown in Figure 20 , where circles represent the fitted Poisson series. The Poisson model proved to be the best fit to the data obtained and was confirmed by χ^2 ($P>0.05$).

For comparison, analysis of the frequency distribution obtained when fish were individually exposed to A.foliaceus larvae (Section II.3.e.ii.page 17) indicates an overdispersed distribution. Of the total 600 larvae exposed to the 30 fish, 301 or 50.17 % of all available larvae became attached. All fish were infected with a mean number of 10.03 larvae per fish (range 1 - 18, variance 40.83, variance to mean ratio 4.0708, χ^2 = 122.1236 significant at $P>0.05$).

The apparant difference in the observed distributions is likely to be related to the experimental regimes used. In the former experiment, a population of fish were exposed to larvae in a single tank and the ratio was 0.01 larvae/fish/litre of water. In the latter experiment, fish were exposed singly to a standard number of larvae. Here the ratio was 0.1 larvae/fish/litre of water. This suggests that under experimental conditions, a low ratio of parasites to fish results in a random distribution, but as the ratio increases, an overdispersed distribution is produced.

(ii) Initial attachment sites

The total numbers of larvae attaching to different sites on G.aculeatus is shown in Table 13 . Analysis of possible differences between the distribution of A.foliaceus on the left or right sides of the body and fins or between the total numbers present on the body and fins/spines is given by χ^2 test. The results indicate that there is no significant difference in the site distribution of A.foliaceus on the left or right sides of the

Figure 20

The experimental frequency distribution

of A.foliaceus on G.aculeatus

Fitted Poisson model

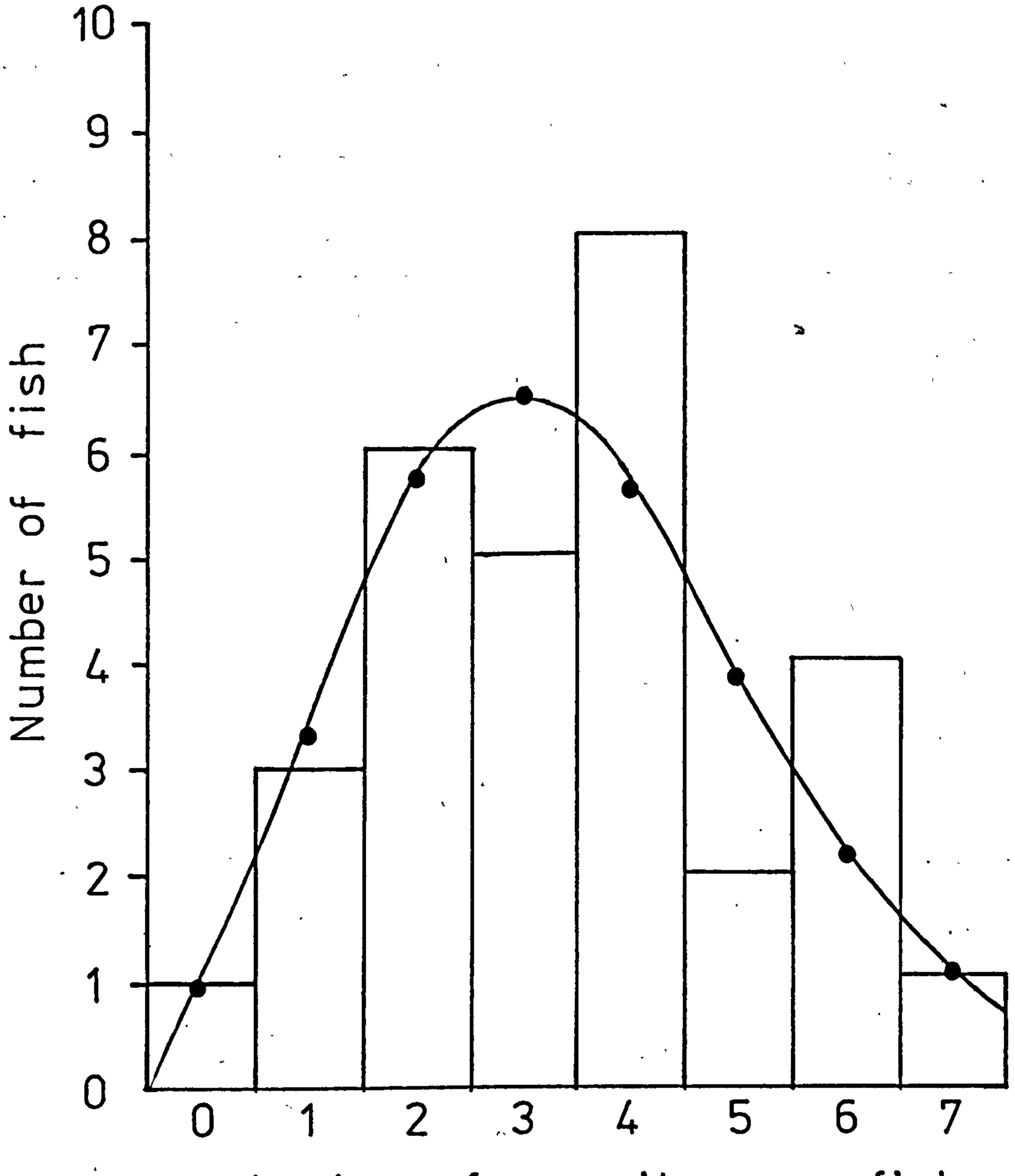

Number of fish
10
9
8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
7
Number of parasites per fish

Table 13

Site distribution of A.foliaceus on G.aculeatus (experimental results)

Site	Total	Position		Chi-square	Significance
		Left	Right		
Fins	116	55	61	0.3103	n/s
Caudal	44	19	25	0.8182	n/s
Anal	17	5	12	2.8824	n/s
Dorsal	20	11	9	0.2000	n/s
Pectoral	35	20	15	0.7143	n/s
Body	136	69	67	0.0294	n/s
Anterior	44	23	21	0.0910	n/s
Middle	55	26	29	0.1636	n/s
Posterior	37	20	17	0.2432	n/s
Ventral	14	-	-	-	-
Dorsal	12	-	-	-	-
		Ventral	Dorsal		
Spines	23	16	7	3.5217	n/s
		Body	Fins + spines		
Total attached	301	162	139	1.7575	n/s

Null Hypothesis is that there should be no significant difference between the attachment of A.foliaceus on either the right or left sides of G.aculeatus.

body and fins. The difference between the total numbers attached to the body and fins/spines is also not significant. The results therefore suggest that the initial attachment of A.foliaceus is random and that all areas on the fish are equally susceptible to attack. Comparison of both field and experimental data on site distribution expressed as a percentage (Table 14) indicates no significant difference ($P>0.05$). The percentage differences observed between individual fins/spines are largely a function of the surface area available for attachment. Both field and experimental results thus indicate that there is no preferential initial attachment site for A.foliaceus larvae.

(iii) Change in attachment sites

The changes in site distribution and total number of A.foliaceus on crucian carp at 20°C. and 10°C. and on sticklebacks are given in Figures 21 ,22,23, and 24, respectively. The difference between the total numbers on the fish and the total number on the body, indicates the total number on the fins. At 20°C., Argulus reached maturity on fish after 3 - 4 weeks (Plates 1,2,3 and 4). At 10°C., Argulus had not reached maturity after 11 weeks on crucian carp at which point the infection had died out, or after four weeks on sticklebacks, when all the fish had died.

Although the numbers of free living parasites are not indicated, emigration from the fish usually involved subsequent predation by the host. Only in one case, was reinfection observed, that of A.foliaceus on crucian carp at 10°C. after six weeks (Figure 22). However, the experiment did not distinguish between individual A.foliaceus and their movements on or off fish.

Changes in the distribution of A.foliaceus on crucian carp at 20°C. show a distinct movement onto the body within the first two weeks (Figure 21). After 3 - 4 weeks, the majority of A.foliaceus are situated on the body (Plates 1,2,3 and 4). The presence of fungal infection at this stage causes

Table 14

Comparison of the experimental and field data on the site distribution of A.foliaceus on G.aculeatus

Site	Percentage		Chi-square	Significance
	Field	Lab.		
Fins + spines	55.84	46.18	0.9147	n/s
Caudal	14.94	14.62	0.0035	n/s
Anal	7.79	5.65	0.3407	n/s
Dorsal	14.94	6.64	3.1923	n/s
Pectoral	11.69	11.63	0.0002	n/s
Ventral spines	5.19	5.32	0.0016	n/s
Dorsal spines	1.30	2.33	0.2923	n/s
Body	44.16	53.82	0.9524	n/s
Anterior	15.58	21.59	0.9718	n/s
Middle	18.18	18.94	0.0156	n/s
Posterior	10.39	13.29	0.3552	n/s

Figure 21

Figure 22

Change in the site distribution and number of A.foliaceus on C.carassius during development at 20° C.

Total number on fish ●——●

Total number on body ●---●

Change in the site distribution and number of A.foliaceus on C.carassius during development at 10° C.

Total number on fish ●——●

Total number on body ●---●

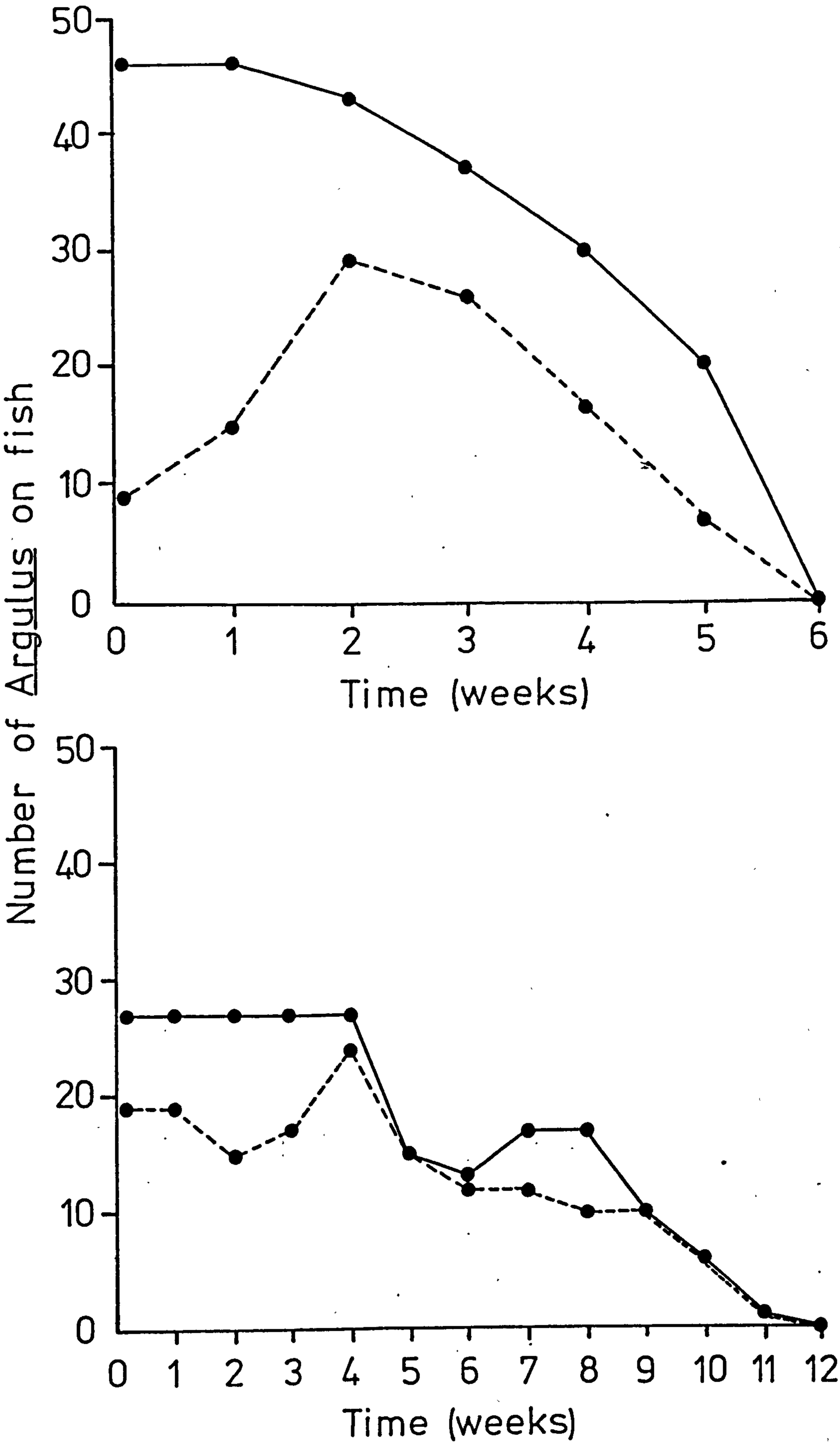

50
40
30
20
10
0
0
1
2
3
4
5
6
Time (weeks)
Number of Argulus on fish
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (weeks)

Figure 23

Figure 24

Change in the site distribution and number of A.foliaceus on G.aculeatus during development at 20° C.

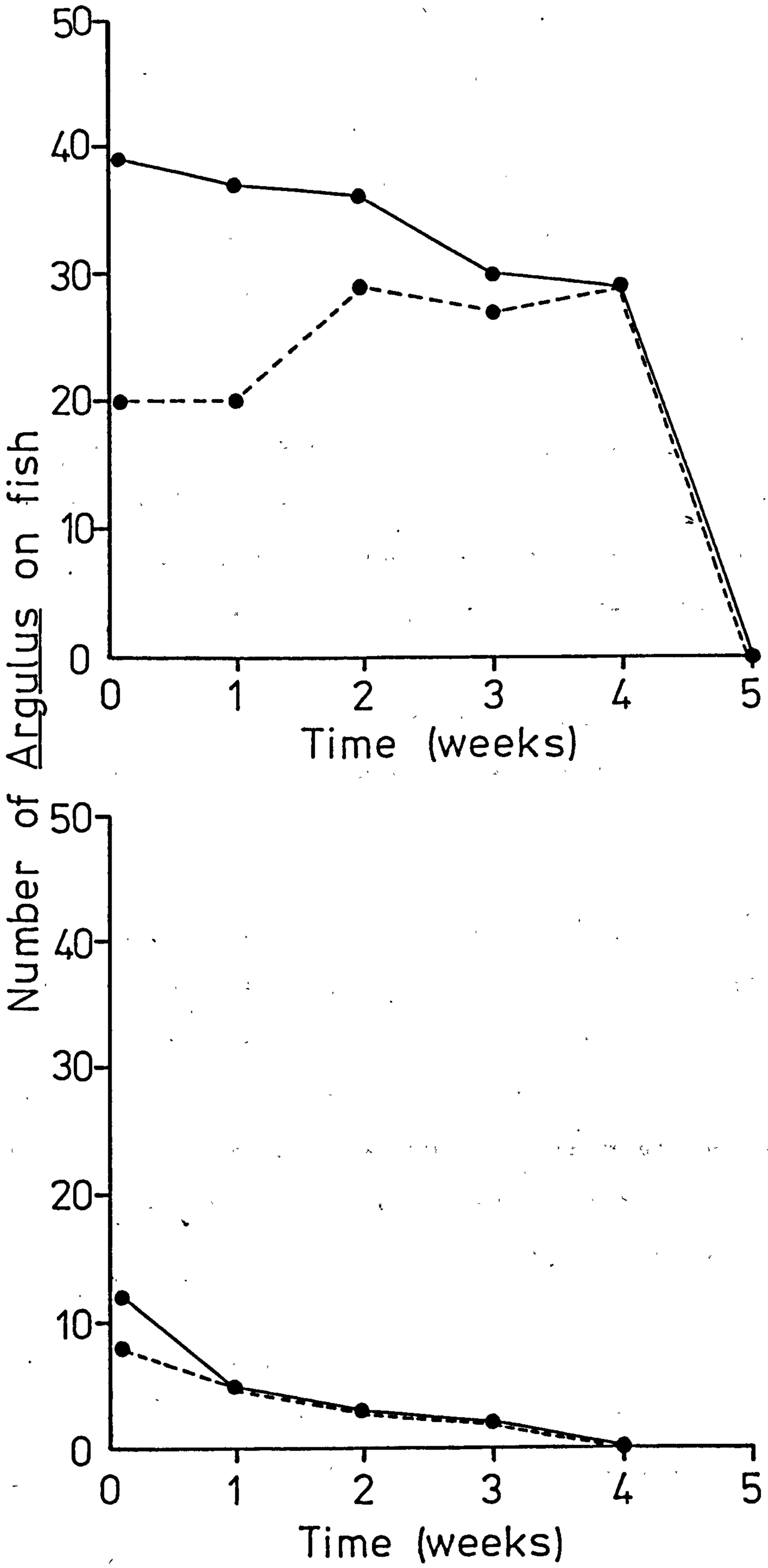
Total number on fish ●——●

Total number on body ●- - -●

Change in the site distribution and number of A.foliaceus on G.aculeatus during development at 10° C.

Total number on fish ●——●

Total number on body ●- - -●


Argulus on fish
50
40
30
20
10
0
0
1
2
3
4
5
Time (weeks)
Number of
50
40
30
20
10
0
0
1
2
3
4
5
Time (weeks)

increased movement of A.foliaceus and aggregation in certain areas (Plates 5 and 6) devoid of fungal growth. Mating activity (Plates 7 and 8) similarly causes increased movement on the fish. After the first week, there is a distinct decline in numbers until the sixth week at which point none of the fish were infected. A similar pattern emerges for A.foliaceus on crucian carp at 10°C., except the initial numbers establishing are much lower and there is no emigration from the fish until the fourth week.

Changes in the distribution of A.foliaceus on G.aculeatus at 20°C. show a similar pattern to that on crucian carp. The sharp decline in numbers after the fourth week was caused by host mortality. At 10°C.,the infection was inherently unstable and died out after the fourth week.

Changes in the number of A.foliaceus on individual crucian carp at 20°C. monitored over a seven day period are given in Figure 25 . The results indicate a distinct daily movement of A.foliaceus between fish during that time and a wide variance in the susceptibility of fish to infection.

(f) Survival

At a laboratory temperature of 10°C., recently hatched Argulus have a maximum life span of approximately nine days with a mean survival rate of 4.5 days. Figure 26 represents the proportion surviving (Nt) against time (t) as a function of the initial number isolated (50). If the instantaneuous death rate were constant, then an exponential decline in population size would be expected (Anderson and Whitfield, 1975). However, the results indicate that the death rate increases with time as a function of age.

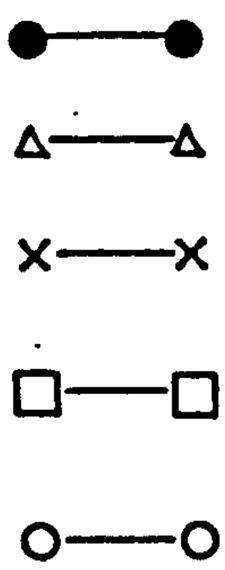
(g) Host species

A.foliaceus was observed to feed on the following fish species: G.gobio, C.carassius, G.aculeatus, P.phoxinus, N.barbatulus, C.auratus,

Figure 25

The movement of A.foliaceus between C.carassius (over a one week period) after all fish were initially infected with a standard density (10 parasites/ fish)

Represent individual fish



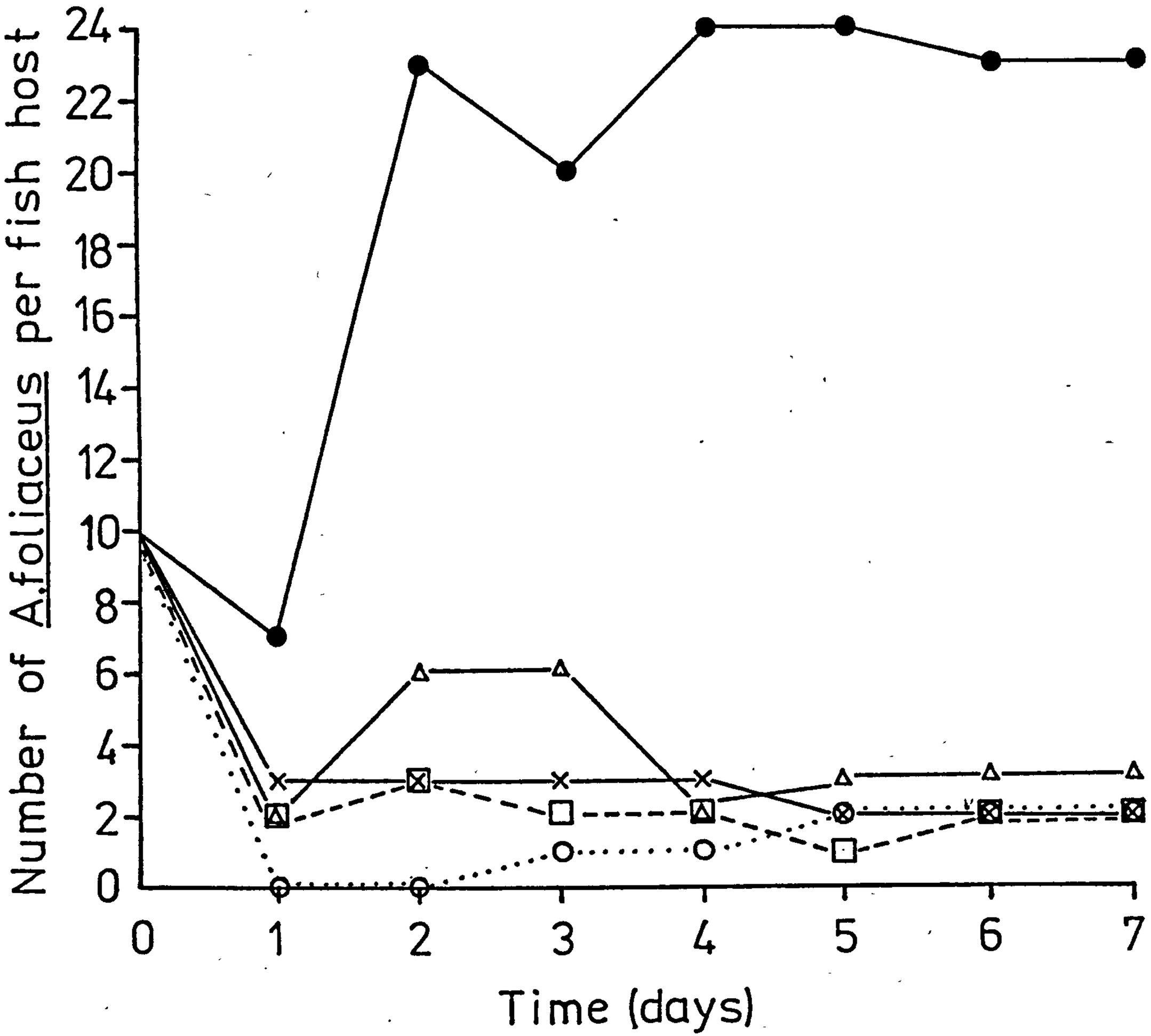
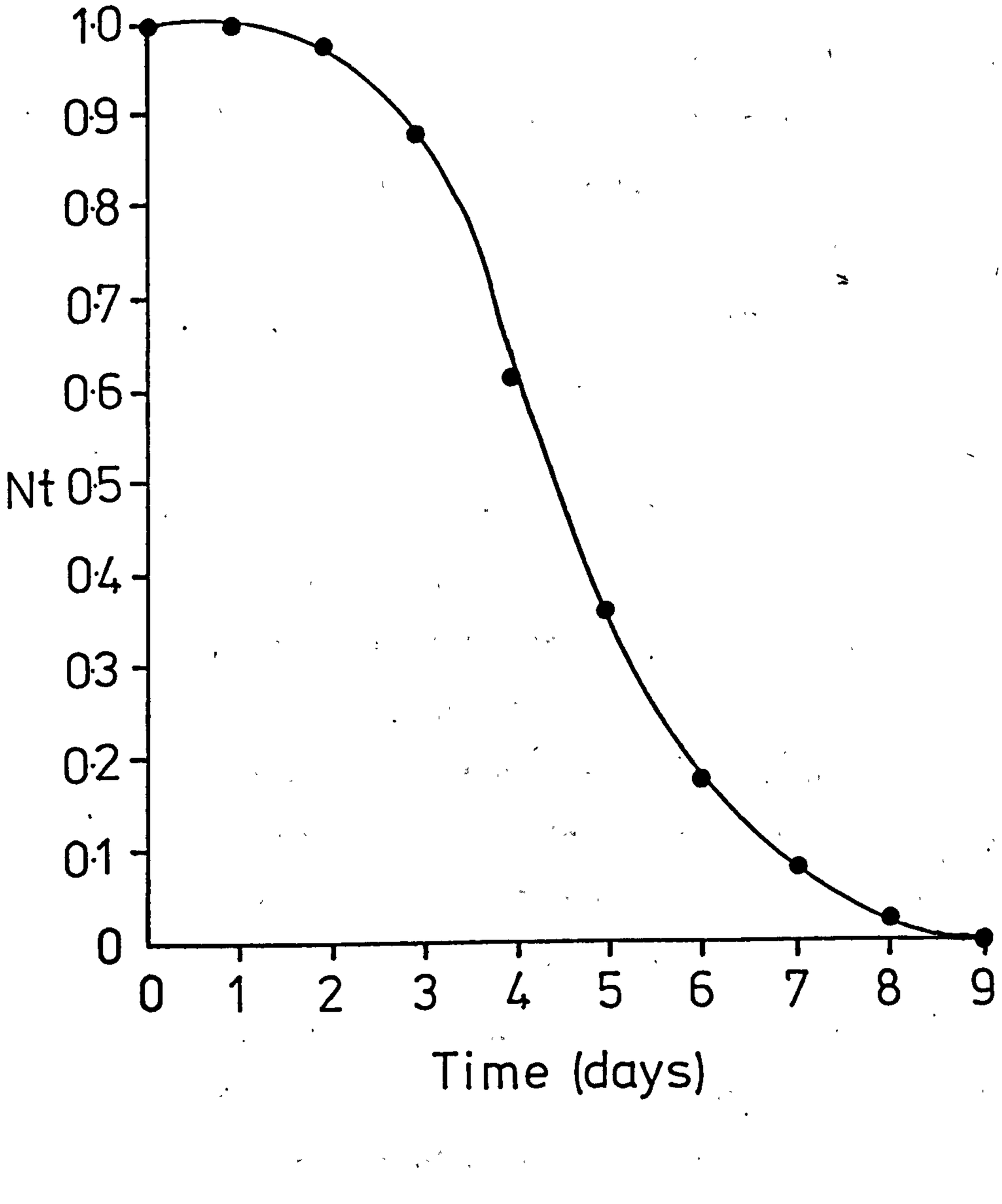

Number of A.foliaceus per fish host
24
22
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Time (days)

Figure 26

The observed number of A.foliaceus larvae surviving at daily intervals, shown as the mean proportion (Nt) of the initial population size


1·0
0·9
0·8
0·7
0·6
Nt 0·5
0·4
0·3
0·2
0·1
0
0 1 2 3 4 5 6 7 8 9
Time (days)

S.erythrophthalmus and P.pungitius. No observations were made for C.gobio or A.anguilla. General estimates only were made on the occurrence of feeding. This was signified by the appearance of blood in the parasite gut and was usually discernible after several hours (Plates 7,8 and 9).

Discussion

The experimental and field studies discussed in this present section illustrate some of the more important factors influencing and controlling parasitism of fish by A.foliaceus.

The seasonal distribution of A.foliaceus on G.aculeatus at Lea Bridge suggests a breeding season from April until October. Larvae hatched in May reach maturity after approximately one month, which is also confirmed experimentally. The decline in the parasite population towards the end of summer might suggest that summer produced A.foliaceus die after breeding. However, this does not account for the possible occurrence of free living A.foliaceus and has to be substantiated by further evidence. The results suggest that an alternation of generations exists amongst the A.foliaceus population, where several new generations appear in the summer. A.foliaceus hatched in late summer may then give rise to an overwintering generation. The difference in the growth rate and development between the winter and summer generations is largely a function of water temperature. Experimental results indicate that at 20°C., maturity is reached after three to four weeks. At 10°C., maturity had still not been attained after 11 weeks. A similar system of alternation of generations has been found in the crustacean parasite Lepeophtheirus pectoralis (Boxshall, 1974b).

The major changes in the sex ratio of A.foliaceus on G.aculeatus (page 31) indicates differences in either natality,mortality or emigration rates between the sexes. However, the increase in the female to male ratio

which begins in September (towards the end of the breeding season) is possibly indicative of a high male mortality rate during the winter. Females surviving throughout the winter produce eggs in the early spring. Under natural conditions, several workers (Herter, 1927a; Bower-Shore, 1940) have also found a higher ratio of females to males parasitic on fish. Kollatsch (1959) found the reverse situation in the free living phase (male to female ratio 3 : 1). He further substantiated these findings in the laboratory. A.foliaceus from cultures developed sexually in equal numbers. When fish were exposed to parasites, a ratio of 2 : 1 females to males infected, whereas a ratio of 1 : 2 females to males was found in the free living phase.

At 20°C., hatching takes approximately four to five weeks and in any one particular batch of eggs, it can take fron up to three to four days for the last larva to emerge. General observation indicates that only a certain proportion of the eggs laid hatch. The survival rate of A.foliaceus follows a pattern similar to that described for the survival of helminth parasites (Anderson, 1976a; Anderson and Lethbridge, 1975; Anderson and Whitfield, 1975). Such age-dependent death rates are usually related to the fact that active, mobile larvae are non-feeding and consume endogenous energy reserves. The death rate is likely to increase as these limited reserves become utilised (Anderson and Whitfield, 1975). It is possible that a similar process occurs in larval A.foliaceus.

Age-dependent activity is known to influence the infectivity of cercariae and miracidia during their respective infection phases, when a decline in infectivity occurs with age (Anderson and Whitfield, 1975; Anderson, Whitfield and Mills, 1975). Although not investigated in this present work, it seems reasonable to assume that the infectivity of larval A.foliaceus would similarly be influenced by such age-dependent activity. All larvae used in the present experiments were therefore approximately one

day old. General observation on free living adults indicates a maximum survival of up to three weeks, but this is likely to be influenced by temperature and nutritional status (although not proven in this present work).

Although searching A.foliaceus larvae and adults show a rheotactic response when in the vicinity of the host, other responses may increase the probability of entering into the host's habitat. Such, however, have not as yet been demonstrated. The reaction to water currents produces increased activity and A.foliaceus "shoot" in the direction of the host (Herter, 1927a). General observation of the response of adults to sticklebacks shows an effective range of approximately 20 cm. This may be influenced by the size and behavioural activity of different fish species and the relative strength of the water currents produced. Larval A.foliaceus usually exhibit this reponse towards fish, but in adults, it is largely dependent upon other free living activities such as mating, egg laying and whether or not the parasites are actually searching for a host. Location of the host may also be influenced by movement of fish towards A.foliaceus (which may provide a potential food source) (Bower-Shore, 1940; Bauer, 1958a) or away from A.foliaceus (Present study).

Certain basic premises were initially accepted with regard to the searching activity of A.foliaceus during the infection phase. It is accepted that the basic strategy of recently hatched larvae is to search immediately for hosts, but this may not necessarily apply to free living adults. Various factors such as egg laying, mating activity, nutritional status, movement of A.foliaceus between hosts (possibly caused by intraspecific competition leading to emigration), poor condition or death of hosts will all contribute to the abundance of A.foliaceus adults in the free living phase. Consequently, it cannot be assumed that the basic strategy of adults in the free living phase is only one of searching for hosts as other

activities will effectively partition the searching time available for infection. In this sense, the foraging period (searching, attack and parasitic phase) of A.foliaceus is somewhat analagous to invertebrate predators where the initiation of the foraging period is commensurate with other biological activities (e.g. mating) in relation to nutritional status (Schoener, 1971; Emlen, 1973).

In studying the infection phase of A.foliaceus, the nature of the experimental regime was as rigidly controlled as possible to provide constant light, temperature, habitat, volume and homogenous populations of both fish and parasites with respect to size, age, sex and nutritional status. Although this regime was initially adopted to investigate larval infections, it is postulated that heterogeneity amongst the searching adult population as well as possible heterogeneity amongst the fish population in their susceptibility to attack could strongly influence the results (in relation to the interpretation of the nature of adult infection). While these factors contributing to heterogeneity are likely to be important in determining infection, they pose problems with respect to the design of experiments concerning adults. The study of first attack tends to isolate certain factors. For example, with no prior exposure to parasite infection, the possibility of host behavioural reaction as a factor influencing successive attack is avoided. Secondly, allowing for heterogeneity in the system (which probit analysis takes into consideration) it is possible to study with precision, the effect of both host and parasite density upon the searching efficiency of adult parasites. Thus, it is possible to determine whether the searching efficiency of adults is affected in the free living phase immediately preceding infection. Such analysis distinguishes between factors which might influence the transmission process once infection is complete (e.g. host behavioural reaction and carrying capacity).

First attack was studied for adult A.foliaceus because of the aforementioned problems and also for ease of observation. Allowing for heterogeneity of searching parasites (and assuming constant experimental parameters), then a simple hypothesis can be formulated about the relationship between first attack and parasite density. If the searching capacity of individual parasites is not affected by increases in parasite density (i.e. influenced by interference/competition), then it could be postulated that the mean time to first attack would decrease exponentially at a constant rate proportional to the parasite density. Considering a single host, if ED 50 (p) represents the mean time to first attack at a given parasite density (p), then the following equation represents the rate of change of ED 50 (p) with respect to parasite density:-

$$\frac{d\ ED\ 50\ (p)}{dP} = - \propto ED\ 50\ (p) \qquad (13)$$

This equation can be expressed as:-

$$ED\ 50\ (p) = ED\ 50\ (0)\ \exp\ (-\propto p) \qquad (14)$$

First attack by both female and male A.foliaceus onto a single host closely follows this form of exponential relationship (page 33). In both cases, the rate of the mean time to first attack is exponentially proportional to the parasite density. The $\propto$ constants for female and male A.foliaceus (0.0811 and 0.0804 respectively) are approximately the same suggesting that the rate of mean time to first attack is constant for both sexes at any given parasite density.

The exponential relationship for a single host can be extended to consider increases in host density. If increasing host density does not influence the searching capacity of A.foliaceus then the net rate of the mean time to first attack within a population of fish (H) can be expressed as:

$$ED\ 50\ (p) = ED\ 50\ (0)\ \exp\ (-\propto Hp) \qquad (15)$$

where ED 50 (p) represents the mean time to first attack on the first host.

Increasing host density is therefore equivalent to increasing parasite density (for example, the mean time to first attack by 40 females onto one host is equivalent to the mean time to first attack by 20 females onto two hosts.). If the exponential relationship obtained for females onto a single host is applied to equation (15), then with increasing host density, the predicted relationship is approximated (Figure 10,page 33). This again suggests that the rate of the mean time to first attack is exponentially proportional to the host density at any given parasite density.

It is of some relevance to compare the A.foliaceus/host interaction with other types of relationship. Studies on predation and insect parasitism (Section I. page 4) have shown that reduction in searching efficiency in relation to increasing predator/parasitoid density may either be caused by mutual interference between searching predators/parasitoids or when a parasitoid encounters a host which has already been parasitised. Searching efficiency can be measured at the simplest level by estimating the number of prey eaten or the number of hosts parasitised over a given time period. Essentially, only one prey/host is available per predator/parasitoid (unless superparasitism occurs) and estimates of the number of prey/hosts attacked during the foraging period are fairly easy to measure. In host/parasite systems, a basic difference is apparant in that at the simplest level, one fish host is available for any number of parasites (assuming that the carrying capacity is not reached). Thus evidence for mutual interference between searching infective stages (during transmission onto a host) is difficult to assess. Various factors such as host response, carrying capacity of the host, competition between parasites on the fish host or heterogeneity amongst the fish or searching parasite populations maybe misinterpreted as interference between searching parasites.immediately prior to infection.

Recent studies on the infection of miracidia onto their snail hosts

(Kuris, 1973; Wilson and Taylor, 1977) investigated the relationship between percentage infection of the host population and miracidial density. They suggested that the reduction in infection observed at high parasite densities could be caused by mutual interference between searching parasites. As pointed out by Anderson (1978b), the apparant relationship between percentage infection and exposure density is in fact related to the distribution patterns of parasites amongst the host population, caused by heterogeneity in their susceptibility to attack and is therefore unlikely to be caused by mutual interference.

Analysis of first attack thus isolates whether mutual interference does occur during the searching phase. It essentially describes an analagous situation to predator/prey and host/parasitoid systems where only one host is available per parasite. It is possible that increased avoidance reaction by the host to increasing parasite density could influence first attack. However, under experimental conditions, avoidance by the fish to some parasites could equally bring the fish into increased contact with other parasites. The spatial distribution of A.foliaceus is therefore an important factor. Aggregations of searching A.foliaceus have not as yet been demonstrated. It is therefore suggested that mutual interference between searching A.foliaceus prior to infection is not a controlling factor in the transmission phase of adults. Similarly, host density does not appear to influence first attack.

Essentially, the dynamics of the A.foliaceus population upon a single host can be described by an immigration-death process which has been applied to other host/parasite systems (see for example, Anderson, 1974; 1976a; 1978b; Anderson, Whitfield and Mills, 1977) (see Section VI, page 90). The present section concentrates mainly upon the immigration (transmission) phase of both adult and larval A.foliaceus. This was investigated in two ways. The first approach studies the influence of parasite density on the

infection of adults over a long time exposure period (12 hours) and the second approach studies the effect of both parasite and host density on the infection of both adults and larvae over a short time exposure period (10 minutes).

Experimental studies have recently identified some of the more important controlling factors influencing the infection rate of certain parasitic stages (Anderson, 1978b; Anderson, Whitfield and Mills, 1977; Anderson, Whitfield and Dobson, 1978; Anderson, Whitfield, Dobson and Keymer, 1978; Keymer and Anderson, 1979). It is relevant to consider whether similar principles apply to the infection of A.foliaceus. Under the experimental conditions described previously (page 15), it is postulated that the infection pattern of both adult and larval A.foliaceus onto a single host could be influenced by two factors, namely parasite density and predation. In the present experimental regime, only predation upon larvae was ever observed. The mathematical model used to investigate A.foliaceus infection has been described previously (Section II.4. page 25). This pattern of infection can theoretically be described for both adult and larval A.foliaceus over any fixed time exposure. In the case of larvae, there is the assumption that the predation rate is constant and unaffected by changes in parasite density. Such predation rates have been discussed for cercariae of T.patialense (Anderson, Whitfield, Dobson and Keymer, 1978). Although predation of larvae was observed, detailed analysis of the predation rate was not determined. Until further experiments can substantiate such rates, it will be accepted that the rate is constant.

Firstly, adult infection will be discussed for both time exposures in relation to changes in parasite density. Over a long period of exposure, similar patterns of infection are observed at all densities of male and female A.foliaceus (Figures 11 and 12). If the infection patterns were to

follow equations (9) and (10), then it would be expected that at any given density (Mo), eventually all parasites would infect the fish. However, infection stabilises after approximately two hours and there is always a residual population of free living A.foliaceus. In order to ascertain whether the rate of infection (α) is constant, equation (10) can be rearranged as the following:-

$$\alpha = -\frac{1}{t} \log_e \frac{(Mo - Pt)}{Mo} \qquad (16)$$

where Mo = original free living A.foliaceus density

Pt = number of A.foliaceus infecting after time t

Over a 12 hour period, at each given density, α is not constant and decreases with time, indicating that the rate of infection is slowing down. The relationship between Pt, Mo, t and α at all densities and sex of Argulus and species of fish is given in Appendix 2. As an example, comparison of the observed and expected rates of infection for five female A.foliaceus are given in Appendix 2 and Figure 27.

Although the rate of infection declines with time, it is still theoretically possible that after any given time exposure, that the number of A.foliaceus infecting (Pt) is directly proportional to the parasite density (Mo). If this were true, then the relationship between (Pt) and (Mo) would be linear and the rate of infection (α) would be equal for each density. After 12 hours, the infection pattern of female A.foliaceus (Appendix 2, Figure 28) indicates a linear relationship at lower densities, which tends towards a plateau at high densities. Thus, at lower densities, α is approximately equal and declines at high densities. At this stage, infection is limiting, because the carrying capacity of the fish has been reached.

Comparison between the infection patterns of both larvae and adults after a short time exposure (10 minutes) in relation to parasite density

Figure 27

Observed and predicted rates of infection

by five female A.foliaceus onto G.aculeatus

△ = Observed rate

○ = Predicted rate

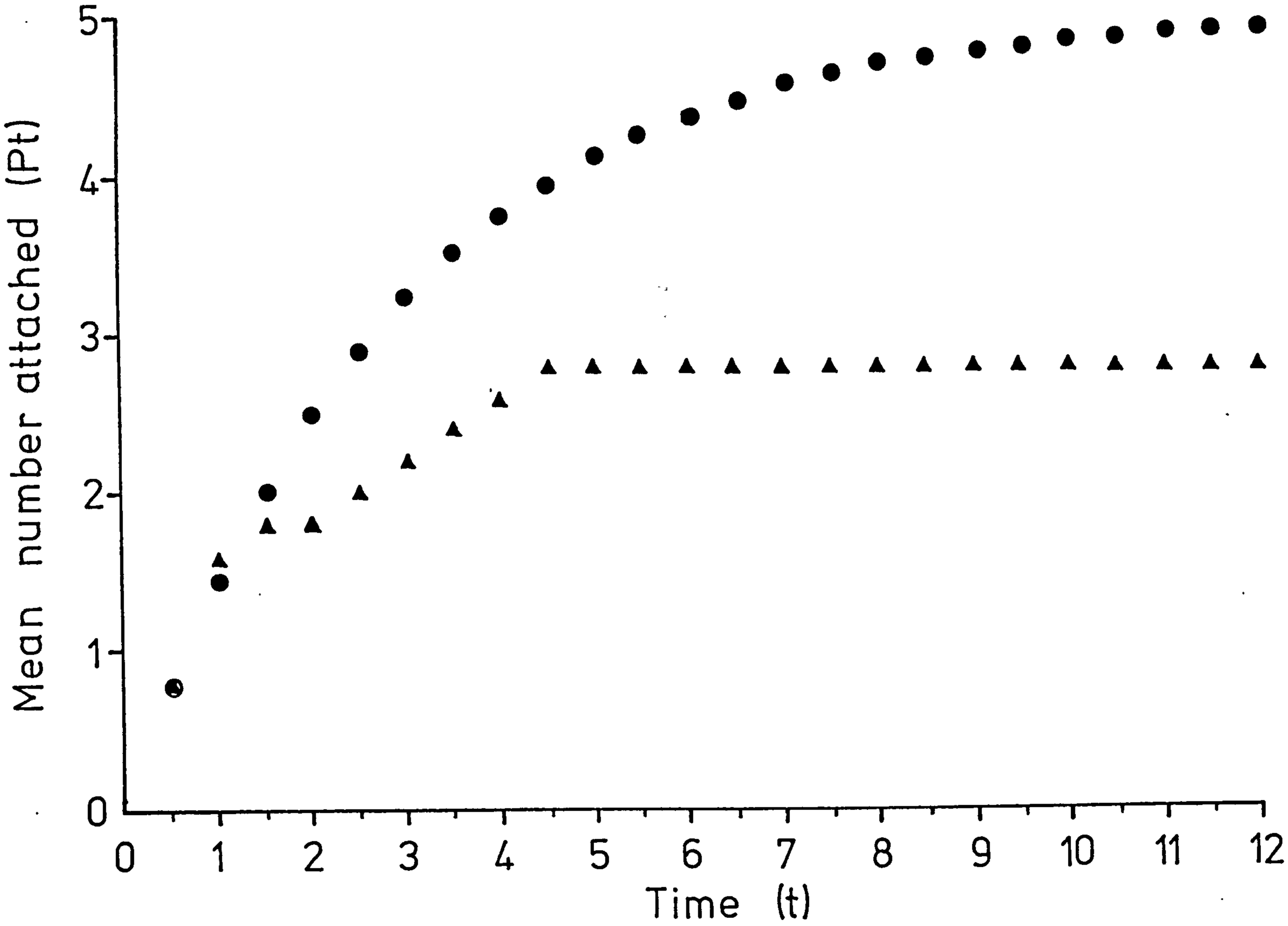
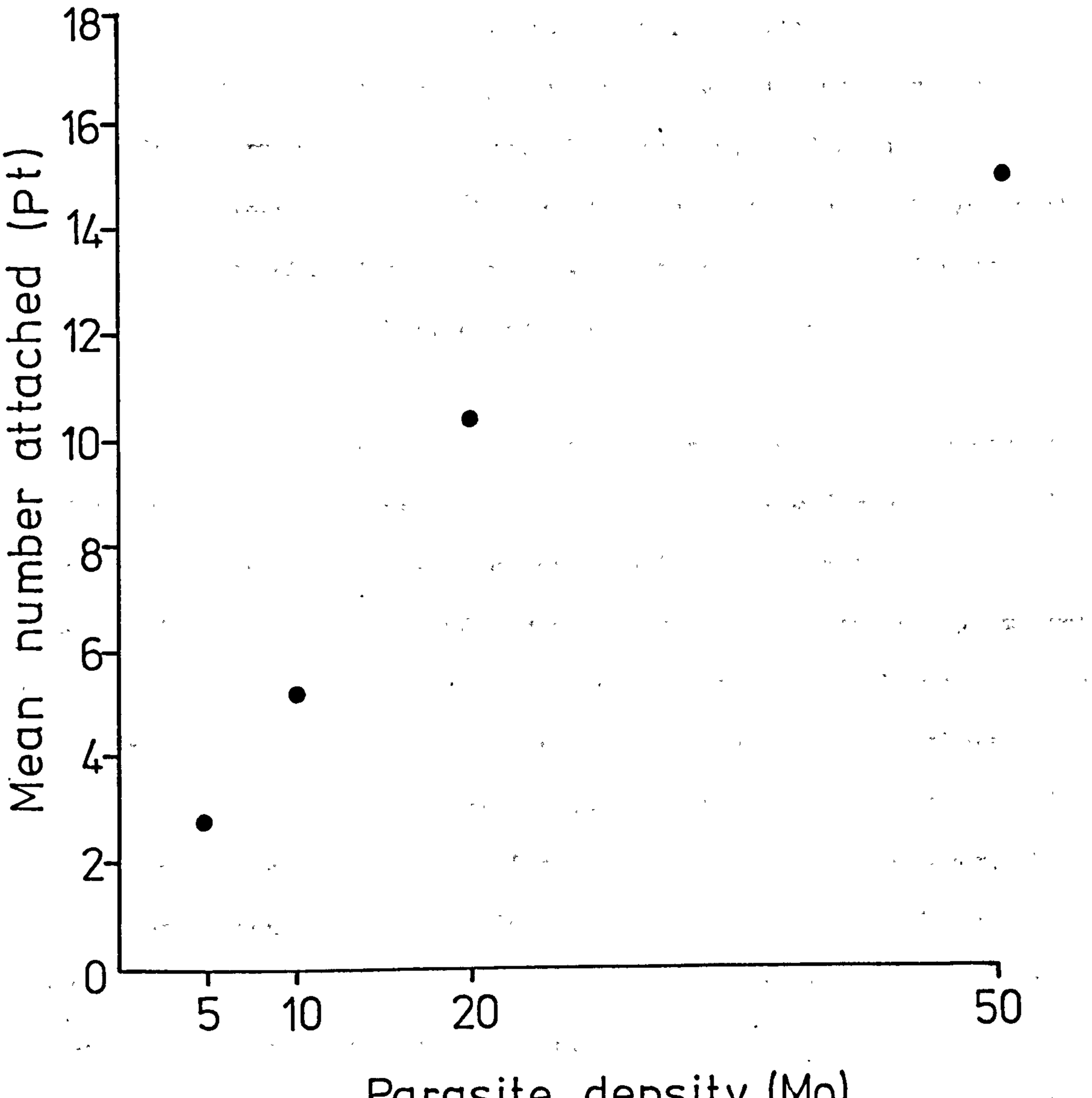

Mean number attached (Pt)
0
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (t)

Figure 28

The relationship between the mean number of A.foliaceus attached (Pt) and parasite density (Mo)


Mean number attached (Pt)
0
2
4
6
8
10
12
14
16
18
5
10
20
50
Parasite density (Mo)

(Figures 15 and 16), indicates several features. The infection pattern of larvae onto a single fish host shows a different relationship from that of adults. The relationship between the mean number attached (Pt) and the parasite density (Mo) (Figure 16) is approximately linear. This indicates that the rate of infection is constant and therefore unaffected by changes in parasite density. Similar patterns have also been observed for miracidia and cercariae, where the rate of infection is directly proportional to the density of infective stages (Anderson, 1978b). In adult A.foliaceus, the infection pattern is approximately linear at low and high densities, but increases in intermediate densities. This is indicated by a sigmoidal relationship between Pt and Mo (Figure 15), and indicates a change in the rate of infection (α). Comparison between the relationship of α and parasite density for both larvae and adults is given in Appendix 2 and Figure 29).

One of the basic premises established is that the strategy of free living adults is not necessarily one of searching for hosts. It is to some extent commensurate with other free living activities in relation to the nutritional status of individual parasites. Heterogeneity amongst the free living population in their response to fish will therefore control the number of available searching parasites. At any density, there should therefore be a residual free living population which does not respond to the fish host, thus the rate of infection will slow down. However, the residual population is likely to decline over a long period of time as nutritional demand increases and searching for hosts becomes the major strategy of the free living population.

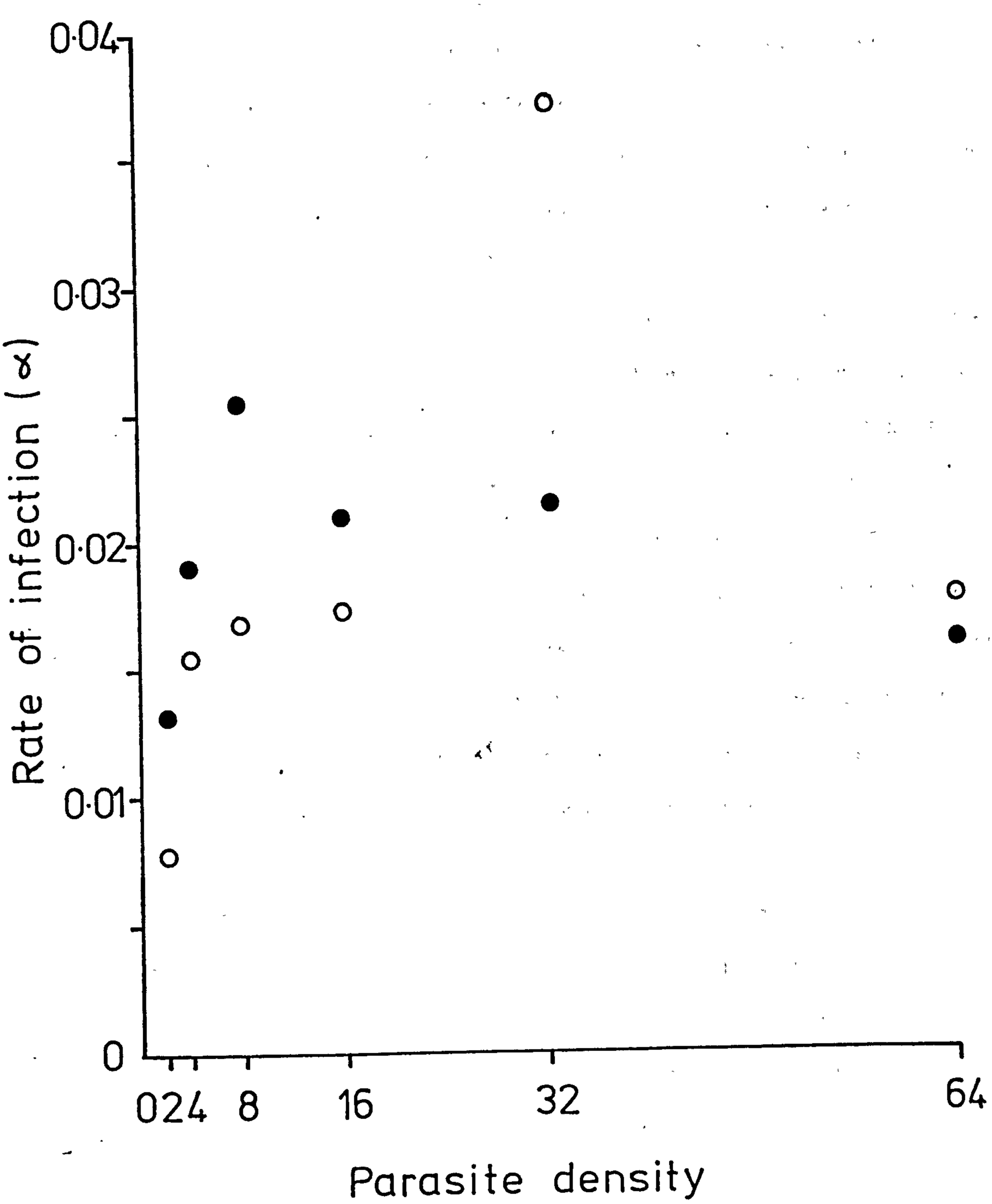
These formulations for adults were based on the investigation of both first attack and patterns of infection over a long time exposure. In both instances, no evidence was present to suggest that mutual interference

Figure 29

The relationship between the rate of infection (α) and parasite density for both larval and adult A.foliaceus

O = Adults

● = Larvae


0·04
0·03
0·02
0·01
0
Rate of infection (α)
024
8
16
32
64
Parasite density

between parasites influenced infection. However, in both cases, the infection experiments represented a somewhat artificial situation in that single sex populations were used. This was primarily to investigate the possible differences in the infection patterns of each sex. Secondly, it was considered that mutual interference could involve two components:-

(a) increased bodily contact (e.g. "bumping or collisions" between parasites) of single sex populations which at high densities could lead to a reduction in searching efficiency.

(b) increased mating activity between mixed sex populations which could also lead to either an increase or a reduction in searching efficiency.

The study of first attack and long time exposure only involved the investigation of (a), whereas adult infection over a short time exposure could include both components. Unfortunately, both available time and material did not allow for an investigation to be completed on short time exposure of single sex adult populations. It has been considered that larval infection is apparently uninfluenced by changes in parasite density. However, in adults, some factors appeared to influence the infection levels reached at different densities. At both low and high levels, infection is approximately proportional to density and could be predicted if no controlling factors were operating. However, at intermediate densities, it is difficult to assess the effects. It has been assumed that A.foliaceus mating in the free living phase could reduce the searching efficiency of adults. However, it is tempting to speculate that mating activity could also lead to an increase in infection, if the females attack the fish whilst the male is attached.. It is postulated that increased parasite density could increase the probability of male/female contact in the searching phase which then leads to increased infection. Again, at very

high levels, the carrying capacity of the fish is reached leading to an apparent decline in infection. Further detailed observations are required to study the precise nature of mixed sex interactions.

The mathematical model used to investigate the effect of host density upon A.foliaceus infection has been described previously (Section II.4. page 26). Since larval experiments with increasing host density were carried out in different volumes of water from that onto one host, and because A.foliaceus adult infection does not follow a constant rate, it is felt that further studies are required to investigate the effect of increasing host density in more detail. Such increases would also have to consider the possible effects of host distribution, reaction of parasites to increased host response and to differences in the predation rate of larvae.

Infection by adults on different host species (over a 12 hour period) indicates that additional factors maybe operant in determining the relative level of the residual free living population. The initial attack of A.foliaceus shows a very slow response to bottom dwelling fish such as N.barbatulus in comparison with mid water swimmers such as C.carassius, P.phoxinus and G.aculeatus. This difference is further exemplified during the infection phase over a 12 hour period. As with those infection rates previously discussed (page 47), the rates are not constant (Appendix 2). The level of the residual population changes as a function of increasing response to "preferred" fish species. Thus, while the initiation of the parasitic phase maybe determined by nutritional demands, the infection levels reached are further determined by the "choice" of host species available. The final levels reached perhaps also reflect the relative surface area available for attachment. Thus C.carassius is more heavily infected than the smaller fish G.aculeatus and P.phoxinus.

One aspect of the population biology of host/parasite interactions is concerned with the distribution of parasites within the host population (See Section I. page 5). Natural distributions of parasites within the host population are generally overdispersed (See for example, Kennedy, 1975a). Although natural parasite dispersion patterns are well documented, very few laboratory studies have investigated the possible generative mechanisms of parasite distribution within the host population (Anderson, Whitfield and Dobson, 1978). While overdispersion can be caused by a number of factors, it is generally held that two basic mechanisms involved are heterogeneity amongst the host population and differences in exposure to infection (Kennedy, 1975a; Anderson, Whitfield and Dobson, 1978).

Little information is available on the distribution of A.foliaceus amongst natural host populations. Quantitative measurements are unreliable because of the tendency for parasites to leave the fish during sampling (Rizvi, 1969; Wootten, 1973; Buckley and Morrice, 1976; Pickering and Willoughby, 1977; Present study). However, the present field studies showed that recently infected fish with larval A.foliaceus exhibited an overdispersed distribution within the host population.

Experimentally, the distribution of adult and larval A.foliaceus upon G.aculeatus shows a similar pattern to that described for cercariae of Transversotrema patialense upon Brachydanio rerio (Anderson, Whitfield and Dobson, 1978). Increases in either parasite density or exposure time leads to an increasing degree of overdispersion within the host population. Increasing either host or parasite density per unit volume of water changes the nature of the frequency distribution from random to overdispersed. The distribution patterns produced by larval A.foliaceus are therefore a consequence of the numbers of searching parasites in combination with differences in host susceptibility. This similarly applies to adults with

the additional factor of heterogeneity amongst the searching population.

Although A.foliaceus is generally considered to be a temporary fish ectoparasite, the precise nature of the host/parasite relationship was imperfectly known. It was generally acclaimed that A.foliaceus leave the host only during the breeding season or upon the death of the host (Wilson, 1902; Bower-Shore, 1940; Bauer, 1958a; Rizvi, 1969). The infection of A.foliaceus on sticklebacks and crucian carp, suggests that other factors may also lead to emigration from fish. It is proposed that the initial distribution of larval A.foliaceus amongst a previously unexposed fish population is largely generated by differences in fish susceptibility. If A.foliaceus initially respond rheotactically to fish movement then the resulting initial distribution is largely caused by variation in fish activity and behaviour. Experimental evidence shows, however, that the distribution of A.foliaceus may change temporally amongst the fish population as a result of movement between hosts. Whether a particular host is then "selected" for will depend upon a number of factors, specifically that of genetic make up, nutritional value and the possible response (behavioural or immunological) by the fish host. At present, little is known of these factors and how they may be related to the infection strategy of A.foliaceus. At the densities of A.foliaceus used in the present experimental studies, competition between parasites upon the fish was not demonstrated. It appears that movement between fish hosts occurs irrespective of the mean parasite burden.

The seasonal change in the distribution of parasites upon the fish population may thus be due to two factors. Firstly, there will be increased recruitment during the summer months as a result of larval immigration. Secondly, there is likely to be a continuous movement of parasites between fish. These factors lead to overdispersed distributions with heavy infection

on more "attractive" fish. Such distributions may partly be explained by differences in fish susceptibility in their "attractiveness" to infection, or by differences in the nutritional status of parasites. Fryer (1966) suggested that settled crustacean parasites attracted others and this could lead to overdipersed distributions. This may also be a realistic possibility for adult A.foliaceus in relation to searching for mates but in larval parasites is harder to explain. Movement between fish maybe costly in time and energy terms and it appears disadvantageous to search for new hosts. This may, however, be related to metamorphosis and feeding where it is necessary to leave the fish between moults. Movement between fish and consequent energy expenditure may also strongly be affected by host species and whether or not shoaling behaviour is exhibited.

It has been suggested that encounters between A.foliaceus and their fish hosts are determined by chance alone and is therefore essentially random. However, once in the vicinity of the host, larvae respond to water currents produced by fish movement. The larval response will be affected by the relative strength of fin or respiratory movements in different fish species. This may then lead to the distribution of larvae in certain areas, depending upon the relative surface area of specific sites. In G.aculeatus, this is not manifested and random site distributions are found.

Unlike many other crustacean parasites, A.foliaceus changes position on its fish host. It is therefore important to distinguish between the initial larval attachment sites and those that are subsequently selected. These temporal changes in site selection maybe affected by fish species (and ease of feeding on different types of scale cover), host susceptibility, search for mates, change in feeding habits as a result of development, secondary fungal infection, competition between parasites and possibly localised tissue/cellular responses.

Under experimental conditions, Kollatsch (1958) noted the movement of larvae away from the fins onto the body during development on Lebistes reticulatus. The present study confirms this on sticklebacks and crucian carp. It is suggested that larvae are not able to penetrate the thick skin or scale cover of different fish species and thus feed on the fins where the blood supply is more accessible. As A.foliaceus mature, they are able to "burrow" beneath scales with the anterior edge of the carapace and penetrate the skin underneath with the proboscis (Wilson, 1902).

Pickering and Willoughby (1977) recorded that perch infected with various fungal species also carried A.foliaceus. Although the lesions and secondary fungal infection may not necessarily by directly attributable to A.foliaceus, they suggested that these branchiurans may act as a vector in transmitting infectious disease. In the present study, it was observed that A.foliaceus movement is strongly influenced by fungal infection and selected sites are those devoid of fungal growth. As the fungal infection develops, emigration from the fish may occur.

When maturity is reached, mating activity also influences the movement of A.foliaceus. This produces aggregation on certain areas of the fish. Since such activities occur on the fish, it is difficult to assess whether particular sites are selected specifically for feeding or act in combination with other factors.

IV. *Piscicola geometra* and *Hemiclepsis marginata*

Introduction

Leech parasitism in fish populations is well known, recent studies being concerned with the potential mortality caused by leeches in fisheries.

In the United Kingdom, some 19 species of leech have been reported on marine fish (Srivastava, 1966), yet only the ecology of *Hemibdella soleae* (Llewellyn, 1965) and *Oceanobdella blenii* (Gibson and Tong, 1969) is well documented.

Some 16 species of freshwater leech are known to occur in the United Kingdom (Elliott and Mann, 1979) but only two parasitise fish. The present study is concerned with some aspects of the biology of these two fish leeches, namely *Piscicola geometra* and *Hemiclepsis marginata*.

Ecological work on the fish leeches is usually restricted to one of three approaches:-

(1) distribution of leeches in the free-living phase.

(2) general reports of their occurrence as parasites.

(3) potential role as vectors in the transmission of protozoan parasites.

It was considered relevant to review the available literature on these three main approaches, since no main comprehensive review has yet brought together the known facts on the ecology of either leech species.

(1) Distribution

In the genus *Piscicola* de Blainville, 1818, some ten species are now recognised (Soos, 1965). *P.geometra* was originally considered to have a Palaearctic distribution, but it has been recorded in North America (Moore, 1898; Bere, 1931; Meyer, 1940, 1946; Oliver, 1958; Klemm, 1972; Davies, 1973) and recently in South America (Soos, 1965).

The genus Hemiclepsis Vejdovsky, 1884 comprises some four species and one subspecies (Mann, 1953). Although H.marginata is recognised to have a Palaearctic distribution (Soos, 1967) it has also been recorded in Africa (Schiacchitano, 1933, 1967).

In Europe, work on the ecology of freshwater leeches is extensive. Factors influencing the distribution of P.geometra and H.marginata as well as other leeches, are well documented (see examples by Pawlowski, 1936a, 1968; Bennike, 1943; Berg, 1948; Lukin, 1962; Wilkialis, 1968; Sandner and Wilkialis, 1972; Khoubkhova and Vojtkova, 1974).

In England, records of the fish leeches indicate a widespread distribution (Brightwell, 1842; Houghton, 1860, 1865; Evans, 1910; Harding, 1910; Robertson, 1912; Whitehead, 1912; Brown, 1935; Mann, 1955a, 1964; Price, 1965; Elliott and Mann, 1979). In Scotland however, only one record has been confirmed for P.geometra (Mills, 1967) and H.marginata is sparse (Warwick and Mann, 1960; Warwick, 1961; Maitland, 1963). Although H.marginata was thought to be rare in Ireland (Harding, 1910; Southern, 1913; Mann, 1964), both leeches are in fact well established (McCarthy, 1975).

It is generally agreed that the distribution of P.geometra is confined to aquatic habitats where the water is in constant motion (Mann, 1955a, 1955b; Wotjas, 1959). This includes fast flowing rivers and oligotrophic lakes (Matysiak, 1967; Mills, 1967; Needham, 1970) where submergent vegetation is available for colonisation (Berg, 1948) and also in eutrophic conditions on reeds by the perimeter of ponds (Dyk, 1963).

By comparison, H.marginata is generally confined to eutrophic conditions in lakes, ponds and marshes or in the lower reaches of slow moving rivers (Mannsfield, 1934; Pawlowski, 1936a; Bennike, 1943; Sandner and Wilkialis, 1972). However, Warwick and Mann (1960) recorded H.marginata in oligotrophic and dystrophic ponds and lakes.

In the British Isles, Mann (1955a) found P.geometra only upon stones, yet it can commonly occur upon Veronica beccabunga and occasionally upon Ranunculus fluitans, Callitriche platycarpa and on Carex sp. (Harrod, 1964). Foreign workers have also found a wide distribution of this leech upon both submergent and emergent vegetation (Pawlowski, 1936a; Bennike, 1943; Berg, 1948; Dyk, 1963; Terekhov, 1968). The latter suggests that the presence of vegetation enhances leech attack upon fish, since fish shoals often move into such areas during feeding and spawning.

H.marginata is found underneath stones (Brown, 1935; Maitland, 1963) and also in and on the leaf blades and axils of various submergent and emergent plants, such as Scirpus sp., Phragmites sp., Typha sp., Stratiotes sp., Potamogeton sp., Nymphaea sp. and Iris pseudacorus (Mannsfield, 1934; Pawlowski, 1936a; Herter, 1937; Needham, 1970; Canning et al., 1973).

P.geometra occurs mainly in soft and intermediate waters and occasionally in hard water (Mann, 1955a; Terekhov, 1968). The pH range varies from 7.0 - 8.4 (Mann, 1955a; Klemm, 1972). H.marginata is usually found in hard, eutrophic waters over a wide range of alkalinities and pH's (6.8 - 9.6) (Mann, 1955a). However, Warwick and Mann (1960) have reported this leech in soft oligotrophic waters.

Bennike (1943) suggested that one reason why P.geometra was absent from small bodies of standing water was because it could not tolerate the higher temperatures reached during the summer. However, because temperature and oxygen concentration are so closely interrelated, it is possible that the oxygen concentration of the water is more important in determining the distribution of this leech (Mann, 1956, 1961). The oxygen consumption of a 30 mg. leech at 20°C. is approximately 10ul/hour and is dependent upon the ambient oxygen concentration between 1 - 6 ml/litre (Mann, 1956). Little is known about the effect of temperature and oxygen concentration

upon the distribution of H.marginata. Physiological reactions to temperature, light, water current and gravity have been investigated (Herter, 1928b, 1929, 1936; Mann, 1962).

In natural conditions, P.geometra can tolerate salinities of up to 8°/oo NaCl (Herter, 1937). In the laboratory, the leech can survive in salinities of 12°/oo for up to 55 days and 18 days at 0°C. and 20°C. respectively (Moller, 1978).

Natural predators of P.geometra are not well documented. Under laboratory conditions, the leech is heavily preyed upon by fish,particularly Gasterosteus aculeatus and cyprinids (Herter, 1937; Terekhov, 1966; Present study). Robertson (1912) noted that adult H.marginata were preyed upon by Nepa cinerea, Dytiscus marginalis, Trocheta subviridis and by fish. Young leeches are attacked by insect and amphibian larvae, Helobdella stagnalis (Herter, 1937) and Glossiphonia complanata (Wilkialis, 1970).

Apart from acting as a vector for Trypanosoma sp. and Cryptobia sp. (= Trypanoplasma sp.) (see Section IV. 3.), H.marginata is also known to be parasitised by the sporozoans Lankestrella minima (= Lankestrella ranarum) and Metamera schubergi (Herter, 1937). It is also an intermediate host for the strigeid digeneans Cotylurus cornutus and Apatemon gracilis (Canning et al., 1973).

Like other leeches, P.geometra is hermaphrodite and cross-fertilization occurs. Reproduction begins early in the spring with an increase in water temperature (Herter, 1937; Bennike, 1943; Mann, 1962; Dyk, 1963) and breeding continues until the autumn (Terekhov, 1967). Sexually mature leeches are distinguished by the presence of a clitellum around the genital opening. During copulation, the bodies of two leeches are entwined and an interchange of sperm occurs. Copulation usually occurs on the vegetation or upon stones. Cocoons are deposited on these substrates two -

three days after copulation (Whitehead, 1912; Brown, 1935; Herter, 1937; Berg, 1948; Dyk, 1963; Matysiak, 1967). At first, they form a whitish-yellow colour, then turn to a dark brown after twenty-four hours (Terekhov, 1966; Matysiak, 1967). Viewed from above, the cocoons are oval. From the side, they are dorsally rounded and ventrally flattened against the surface upon which they are laid. Cocoons are 1.0 - 1.5 mm. in length (Podubsky and Stedronsky, 1954). During deposition of the cocoons, the adult leech fastens both suckers to the substrate. Before the cocoon is released through the clitellum, a fertilized ovum and nutritive material are injected into it from the female gonopore and clitellar glands respectively. The cocoons are fastened to the substrate with a cementing substance (Brumpt, 1900). Each fertile cocoon contains one embryo and development takes approximately 13 - 20 days at 15 - 26°C. Young leeches are about 4 - 5 mm. in length when they hatch (Terekhov, 1966,1967).

The reproductive span of H.marginata lasts from May until September, with an intensive period in June - July (Wilkialis, 1970). Brooding leeches lay eggs in the laboratory approximately three to twenty-six days after copulation (Needham, 1970; Wilkialis, 1970). Eggs are yellow, arranged in two layers joined together by a gelatinous mass and attached to the underside of the adult body. In rare cases, the eggs are deposited on stones or to aquatic plants, but the leech generally incubates them (Sawyer, 1971). Embryos are discernable seven to eight days after the eggs are laid and in a further two weeks, young leeches are distinguishable (Needham, 1970). During this period, the adult leech fans the eggs by undulating movements and after hatching, the young are carried around by the adult on the ventral surface of the body. After several weeks, great activity of the young is noticeable during which, the adult is actively searching for a host. When a host is found, young leeches leave the adult

and pursue independent life. Leeches at this stage are approximately 2.0 - 4.0 mm. in length (Needham, 1970).

(2) Parasitism

The distribution of P.geometra as a fish parasite is well documented (see Table 15).

The majority of published records for fish acting as hosts to P.geometra are from the Palaearctic zone. A list of freshwater fish indigenous to the British Isles (Maitland, 1972) which are known to be hosts for P.geometra is given in Table 15. The list includes both British and foreign records. The latter are included to indicate that many freshwater fish (although not reported as hosts for P.geometra in this country) are nevertheless parasitised by the leech in other Palaearctic regions. P.geometra has also been recorded on the marine fish Cottus scorpius in the British Isles (Knight-Jones, 1962). Twenty fish species which do not occur in the British Isles are known to be hosts for P.geometra (Bykhovskaya-Pavlovskaya, 1964; Pawlowski, 1968; Kulakhovskaya, 1976). It can be seen therefore, that P.geometra exhibits an extremely wide specificity since a total of 56 fish species can act as hosts for this leech. Since the fish hosts for P.geometra are found in a wide variety of habitats, it is conceivable that the leech is able to tolerate a far wider range of conditions than is suggested by the records of its distribution in the free living phase.

As well as being a fish parasite, H.marginata has often been reported to feed on Amphibia (Herter, 1928b, 1937; Pawlowski, 1936a, 1968; Perret, 1952; Mann, 1953, 1962; Wilkialis, 1970) and also Molluscs (Soos, 1967). A list of the occurrence of H.marginata on freshwater fish indigenous to the British Isles is given in Table 16. This includes both British and foreign records using the same rationale as described above

Table 15

Host records for Piscicola geometra

Fish species	Reference
Salmo salar	Mills (1967) McCarthy (1975) * Pawlowski (1968)
Salmo trutta	Price (1965) Mills (1967) Needham (1970) McCarthy (1975) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968) * Borgstrom and Halvorsen (1972)
Salmo gairdneri	Needham (1970) Wootten (1973) * Dogiel et al. (1958) * Bykhovskaya-Pavlovskaya (1964)
Salvelinus fontinalis	* Pawlowski (1968)
Coregonus lavaretus	* Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Thymallus thymallus	Davies (1967) * Pawlowski (1968)
Osmerus eperlanus	* Voigt (1977)
Esox lucius	Mishra and Chubb (1969) Needham (1970) Anderson (1971) Canning et al. (1973) McCarthy (1975) * Gaschoff (1926) * Dyk (1963) * Bykhovskaya-Pavlovskaya (1964) * Halvorsen (1964) * Matysiak (1967) * Pawlowski (1968) * Borgstrom and Halvorsen (1972)
Cyprinus carpio	Needham (1970) Anderson (1971) * Gaschoff (1926) * Dogiel et al. (1958) * Dyk (1963) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968) * Bazal, Lucky and Dyk (1970) * Moller (1978)
Carassius carassius	Anderson (1971) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)

(contd..)

Fish species	Reference
Carassius auratus	* Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Barbus barbus	Needham (1970) * Gaschoff (1926) * Pawlowski (1968)
Gobio gobio	Scharff (1898) Southern (1908) Needham (1970) Anderson (1971)
Tinca tinca	Needham (1970) Anderson (1971) * Gaschoff (1926) * Dogiel et al. (1958) * Dyk (1963) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Blicca bjoerkna	* Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968) * Wierzbicka (1978)
Abramis brama	Needham (1970) Anderson (1971) * Dogiel et al. (1958) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968) * Wierzbicka (1978)
Alburnus alburnus	* Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Phoxinus phoxinus	Needham (1970) * Pawlowski (1968)
Scardinius erythrophthalmus	Anderson (1971) * Terekhov (1967)
Rutilus rutilus	Brightwell (1842) Davies (1967) Mishra and Chubb (1969) Needham (1970) Anderson (1971) * Dyk (1963) * Bykhovskaya-Pavlovskaya (1964) * Terekhov (1967, 1968) * Pawlowski (1968) * Borgstrom and Halvorsen (1972)

(contd..)

Fish species	Reference
Leuciscus cephalus	Davies (1967) Needham (1970) * Bykhovskaya-Pavlovskaya (1964) * Halvorsen (1964)
Leuciscus idus	* Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Leuciscus leuciscus	Davies (1967) Needham (1970) * Halvorsen (1964)
Noemacheilus barbatulus	McCarthy (1975)
Silurus glanis	* Dogiel et al. (1958) * Dyk (1963) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Gasterosteus aculeatus	McCarthy (1975) Present study * Bykhovskaya-Pavlovskaya (1964) * Terekhov (1967) * Pawlowski (1968)
Pungitius pungitius	McCarthy (1975) * Bykhovskaya-Pavlovskaya (1964)
Lota lota	* Pawlowski (1968) * Halvorsen (1972)
Micropterus salmoides	* Pawlowski (1968)
Lepomis gibbosus	* Dogiel et al. (1958)
Perca fluviatilis	Mishra and Chubb (1969) Anderson (1971) * Perret (1952) * Dogiel et al. (1958) * Bykhovskaya-Pavlovskaya (1964) * Matysiak (1967) * Terekhov (1967) * Pawlowski (1968)
Gymnocephalus cernua	Wootten (1973) * Bykhovskaya-Pavlovskaya
Stizostedion lucioperca	* Dogiel et al. (1958) * Bykhovskaya-Pavlovskaya (1964) * Terekhov (1967, 1968) * Pawlowski (1968)
Cottus gobio	Needham (1970) * Pawlowski (1968)
Platichthys flesus	Knight-Jones (1962) * Pawlowski (1968)

* Denotes foreign records

Table 16

Host records for Hemiclepsis marginata

Fish species	Reference
Esox lucius	McCarthy (1975) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968) * Wilkialis (1970)
Cyprinus carpio	* Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Carassius carassius	* Pawlowski (1968)
Carassius auratus	* Pawlowski (1968)
Barbus barbus	* Pawlowski (1968)
Tinca tinca	* Pawlowski (1968)
Abramis brama	McCarthy (1975) * Pawlowski (1968)
Scardinius erythrophthalmus	McCarthy (1975) * Pawlowski (1968)
Rutilus rutilus	McCarthy (1975) * Pawlowski (1936a, 1968) * Bykhovskaya-Pavlovskaya (1964)
Leuciscus idus	* Pawlowski (1968)
Silurus glanis	* Bykhovskaya-Pavlovskaya (1964)
Gasterosteus aculeatus	Letch (1977) Present study * Pawlowski (1936a, 1968)
Perca fluviatilis	McCarthy (1975) * Bykhovskaya-Pavlovskaya (1964) * Pawlowski (1968)
Gymnocephalus cernua	* Bykhovskaya-Pavlovskaya (1964)
Noemacheilus barbatulus	* Pawlowski (1968)
Anguilla anguilla	* Pawlowski (1968)

* Denotes foreign records

for P.geometra. Natural infections of the leech upon amphibia have not been recorded in the British Isles, although foreign records indicate that it occurs on Bufo bufo (Perret, 1952), Rana temporaria, Rana esculenta and Trituris vulgaris (Pawlowski, 1936a, 1936b, 1968). It has also been reported on three fish species which do not occur in the British Isles (Bykhovskaya-Pavlovskaya, 1964; Markov, Reshtnikhova and Trusov, 1965).

In the British Isles, although it is frequently stated that H.marginata is a fish parasite (see for example, Mann, 1955a; Needham,1970) actual records of the leech naturally parasitising fish are rare (see Table 16). Information as to the intensity of infection, sites of attachment or seasonal incidence is not available.

Records of seasonal changes in the occurrence of P.geometra upon fish are somewhat confusing. Bauer (1958a) recorded that leeches appear on fish in autumn and overwinter until early summer on Cyprinus carpio in fish farms. Leeches then leave the fish to deposit cocoons but they reinfect the fish during the second half of the summer. Other workers (Dyk, 1963; Matysiak, 1967; Terekhov, 1968; Needham, 1970; Anderson, 1971; Halvorsen, 1972) have recorded P.geometra on fish at different times of the year. These records make it difficult to ascertain whether or not a seasonal cycle is present.

Harding (1910) and Herter(1929, 1937) both examined the method of attack by P.geometra. Essentially, "hungry" leeches move rapidly onto the upper surface of stones and vegetation when a fish is introduced into the experimental tank. This response is generated by water currents created by the fish. The posterior sucker of the leech is firmly attached to the substrate with the body extended and the anterior end "swaying" in a random movement. When a fish is within reach, the anterior sucker is attached to the fish, the posterior sucker released rapidly and moved into a

position immediately behind the anterior sucker. The anterior sucker is then released in order to search for a suitable feeding area. Although P.geometra is able to swim rapidly, initial attack by swimming has never been observed.

"Hungry" H.marginata react to wave movements in the water by exhibiting a positive rheotactic response in the direction of the fish. Chemoreception is quite well pronounced (Herter, 1928b). Leeches rest on the substrate with the anterior body held in a vertical position. Locomotory movements, shadows and mucus produced by the fish excites the leech to carry out seeking activities. Attack occurs by a similar method to that of P.geometra.

Site selection of P.geometra has been discussed by a number of workers (Brightwell, 1842; Dyk, 1963; Mills, 1967; Bazal, Lucky and Dyk, 1969). Preferential attack sites are the head, abdomen, dorsal, pectoral and caudal fins. Several workers have noted that piscicolids may leave the fish or become dislodged from their original positions during field sampling (Thompson, 1927; Wootten, 1973; Khan and Meyer, 1976). Consequently, accurate estimates of naturally occurring selection sites are difficult to obtain.

Experimental infections indicate that P.geometra feeds on the fins of the fish host (Harding, 1910; Whitehead, 1912; Brown, 1935). Robertson (1912) noted that young H.marginata easily pierce through the scales of goldfish and one preferential site is the external nares. The leech tends to feed on sites first reached after attack, such as the fins, ventral surface and mouth of tench (Needham, 1970). Wilkialis (1970) noted that the majority of leeches would feed on the abdomen, eyes, mouth and around the fins of Cyprinus carpio.

Recently hatched P.geometra remain attached to carp and rudd for

seven to eight hours before leaving to rest on the walls of the aquaria (Terekhov, 1966, 1967). Although the infection time is variable, leeches detach from the fish when feeding is complete (Harding, 1910; Whitehead, 1912; Brown, 1935; Dyk, 1963). Young H.marginata feed on fish for up to six hours and after feeding, are never ready to feed again for about ten days from the time they leave the fish. The time between successive feeds is determined by the size of the leech, the amount of blood ingested and the temperature. At 5°C., survival time without feeding can last up to eight months. At 15°C., young leeches reach maturity after seven feeds (Needham, 1970).

Both P.geometra and H.marginata belong to the suborder Rhynchobdellae. Unlike the Gnathobdellae (where the mouth is a wide aperture occupying the whole of the anterior sucker), the Rhynchobdellids possess a pharynx which is modified to form an everscible proboscis (Mann, 1964). The fish leeches feed by extending the proboscis and piercing the tissues of the host fish. In P.geometra, salivary secretion is discharged onto the host tissues during feeding (Keysellitz, 1906). This secretion probably contains an agent which locally increases the blood supply at the feeding site (Van der Lande, 1968).

Jennings and Van der Lande (1967) have investigated digestion in leeches. In the fish leeches, digestion in the gut lumen results in the breakdown of erythrocytes, lysis of nuclear membranes and release of nuclear constituents. Haemoglobin is broken down to insoluble granules of haematin. In P.geometra, extensive digestion occurs in the crop, where erythrocytes are digested within ten days. In H.marginata however, digestion occurs mainly in the intestine with food being passed into it at intervals from the crop. Jashke (1933) found an albuminous fluid containing large numbers of bacteria in the oesophageal diverticula of P.geometra. These bacteria have

subsequently been identified as Pseudomonas sp. (Jennings and Van der Lande, 1967). These bacteria may be important in leech nutrition in that they supply essential vitamins to the diet.

(3) Leeches as vectors

Both species of leech have long been considered as vectors in the transmission of protozoan parasites to freshwater fish (see Molyneux, 1977, for a review of trypanosome development in leeches). H.marginata has also been proposed as a vector in the transmission of Trypanosoma rotatorium to Rana esculenta (Noller, 1913). Leydig(1857) first recorded the presence of flagellates in the crop of P.geometra. In 1901, Doflein suggested that leeches might transmit these flagellates to fish.

Brumpt (1906a, 1906b, 1906c, 1907), Robertson (1912) and Needham (1970) all successfully transmitted Cryptobia sp. (= Trypanoplasma sp.) into fish by using infected H.marginata. Keysellitz (1906) stated that P.geometra could also transmit Cryptobia sp. into fish, but this has never been confirmed (Needham, 1970). However, in America, other piscicolids are known to transmit Cryptobia sp.(Becker and Katz, 1965, 1966; Putz, 1972).

The transmission of Cryptobia sp. in relation to the feeding activity of leeches is still not clearly understood. Repeated feeding of H.marginata gives rise to heavy infections of Cryptobia sp. in fish (Needham, 1970). Several authors suggest that because P.geometra stays on the fish between blood meals, repeated inoculations of Cryptobia sp. will result in high infections (Keysellitz, 1906; Needham, 1970; Canning et al., 1973). It has been mentioned previously that P.geometra leaves the fish after feeding. Heavy infections of Cryptobia sp. could therefore only arise if a fish was continually reinfected by infective leeches, or by Cryptobia dividing in blood.

H.marginata will also transmit Trypanosoma sp. (Brumpt, 1906a, 1906b, 1906c; Robertson, 1912; Qadri, 1952, 1962; Needham, 1970; Letch,

1977). The experimental development of trypanosomes in P.geometra has not yet been achieved. Leger (1905) noted the development of trypanosomes in P.geometra for only four days, while other workers were unable to transmit trypanosomes to fish by using P.geometra (Brumpt, 1906c; Qadri, 1952; Needham, 1970; Letch, 1979). Molyneux (1977) suggests that H.marginata is the most suitable host for trypanosomes and Cryptobia sp. and that P.geometra has a poor vectorial capacity.

Trypanosomes are considered to be highly host specific. Since leeches feed on a wide range of fish species it has been suggested that minor morphometric differences of trypanosomes are indicative of their polymorphic diversity (Becker and Katz, 1966). Recent studies by Letch (1979) have however, shown that there are no basic morphological or biochemical distinctions between trypanosomes occurring in different fish species and that they should all be classified as one species. This point will be referred to elsewhere (see page 78).

Aeromonas liquefacians has also been isolated from P.geometra. Leeches can transmit hemorrhagic septicaemia to healthy carp within twelve days of infection (Dombrowski, 1953).

Experimental studies

The present section analyses certain aspects of the relationship between P.geometra and its fish hosts.

Initially, a comparative experimental study was intended between the two species of leech and A.foliaceus. The experimental regime, however, was difficult to maintain. The low availability of H.marginata from the field connected with heavy predation and high natural mortality of both species in the laboratory provided insufficient material for numerous replicate experiments. It was also difficult to carry out experiments on

young leeches and eliminate predation without altering the behaviour of fish. Where possible, comparative studies were made between P.geometra and A.foliaceus. However, emphasis was placed on the more important features of the parasitic phase of both leech species, particularly with respect to the method of attack, feeding and host specificity.

Materials and methods

See Section 11. 1, 2, 3 (a) (b) (d) (e)ii, iii, (f) (g), 4 (pages 7-21).

Results

1. Field sampling

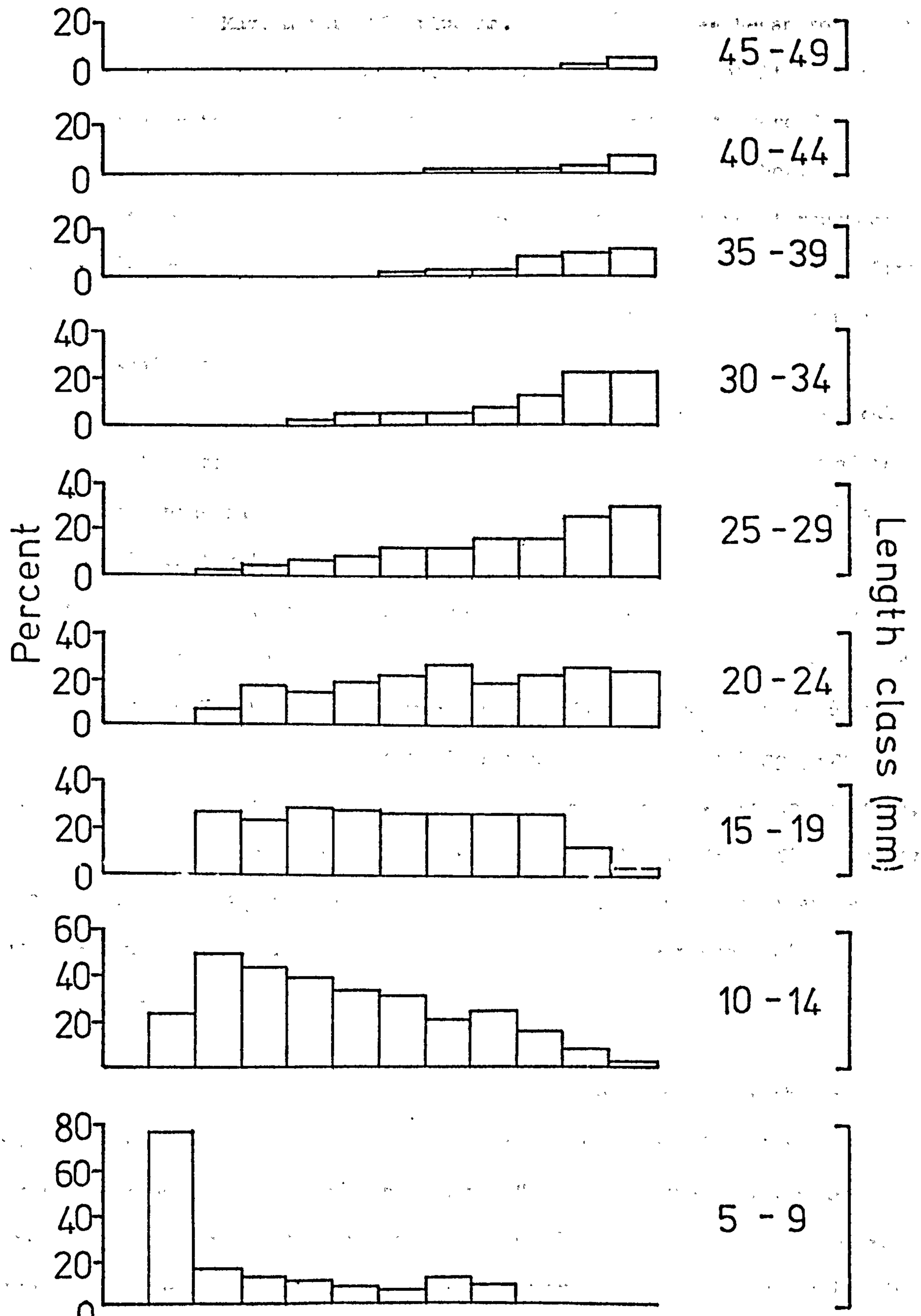
H.marginata sampled at Flatford Mill were collected from the leaf blades and axils of Typha latifolia (L.) and from pieces of driftwood. From March to June, the majority of leeches were mature and brooding eggs, whereas from July to September, mature adults were found with young attached to the ventral surface of the body. No leeches were obtained in the winter months as the reeds had been cut and removed by the Anglian Water Authority. No fish sample was available from Flatford Mill, but H.marginata was found on G.aculeatus at Lea Bridge.

At Flatford Mill, free living P.geometra were found on Ceratophyllum demersum (L.), Myriophyllum spicatum (L.) and Elodea sp. and at Lea Bridge on Nuphar lutea (L.), Potamogeton lucens (L.) and Fontinalis antipyretica (Hedw.). Although quantitative measurements could not be made accurately, it was possible to estimate the change in the percentage numbers of leeches present in each length class per month (Figure 30).

Figure 30

Seasonal change in the length class

structure of P.geometra samples


Percent
Length class (mm)
45 -49]
40 -44]
35 -39]
30 -34]
25 -29]
20 -24]
15 -19]
10 -14]
5 -9]
20
0
40
60
80
M A M J J A S O N D J F
Month

In April, young leeches of between 5 - 14 mm. only were present in the sample and in May, mature (20 plus mm. length) leeches began to appear. During the summer, a steady increase in size was observed but it was not until September that leeches in the 35 plus mm. length class were found. Young leeches (5 - 9 mm.) were present from April until November.

Analysis of the change in the mean length of leeches throughout the year (Figure 31) indicates that the growth rate was rapid in the first month after hatching (April - May), low in the summer and autumn months and high again in the winter (November - February).

The absence of leeches in the March sample maybe due to several factors. It is possibly a sampling error due to the reduction of weed in the winter months and to the small numbers of leeches found during this period. Alternatively, leeches may inhabit other substrates because of the sparsity of weed, or move onto the fish population. Changes in the length frequency distribution suggest that heavy adult mortality may occur in February and March, with the free living population represented as cocoons.

Monthly changes in the incidence and intensity of P.geometra on G.aculeatus are recorded in Table 17. Because of the low number recorded, it is difficult to draw any conclusions from this data. Other fish species such as C.carpio, A.brama, P.fluviatilis and E.lucius also occur at Lea Bridge, but sampling of these fish was beyond the scope of the present study.

2. Infection

(a.i.) Attack (general observations)

The introduction of a fish produces a variable reaction by H.marginata resting on weed or walls of an aquarium. Recently fed leeches or those brooding eggs show no reaction. "Hungry" leeches (i.e. starved for one week) react immediately to the presence of a fish by initially moving the anterior end of the body towards the current produced by the fish. This

Figure 31

Seasonal change in the length frequency distribution of P.geometra.

● = Observed means. Vertical bars represent 95% confidence limits.

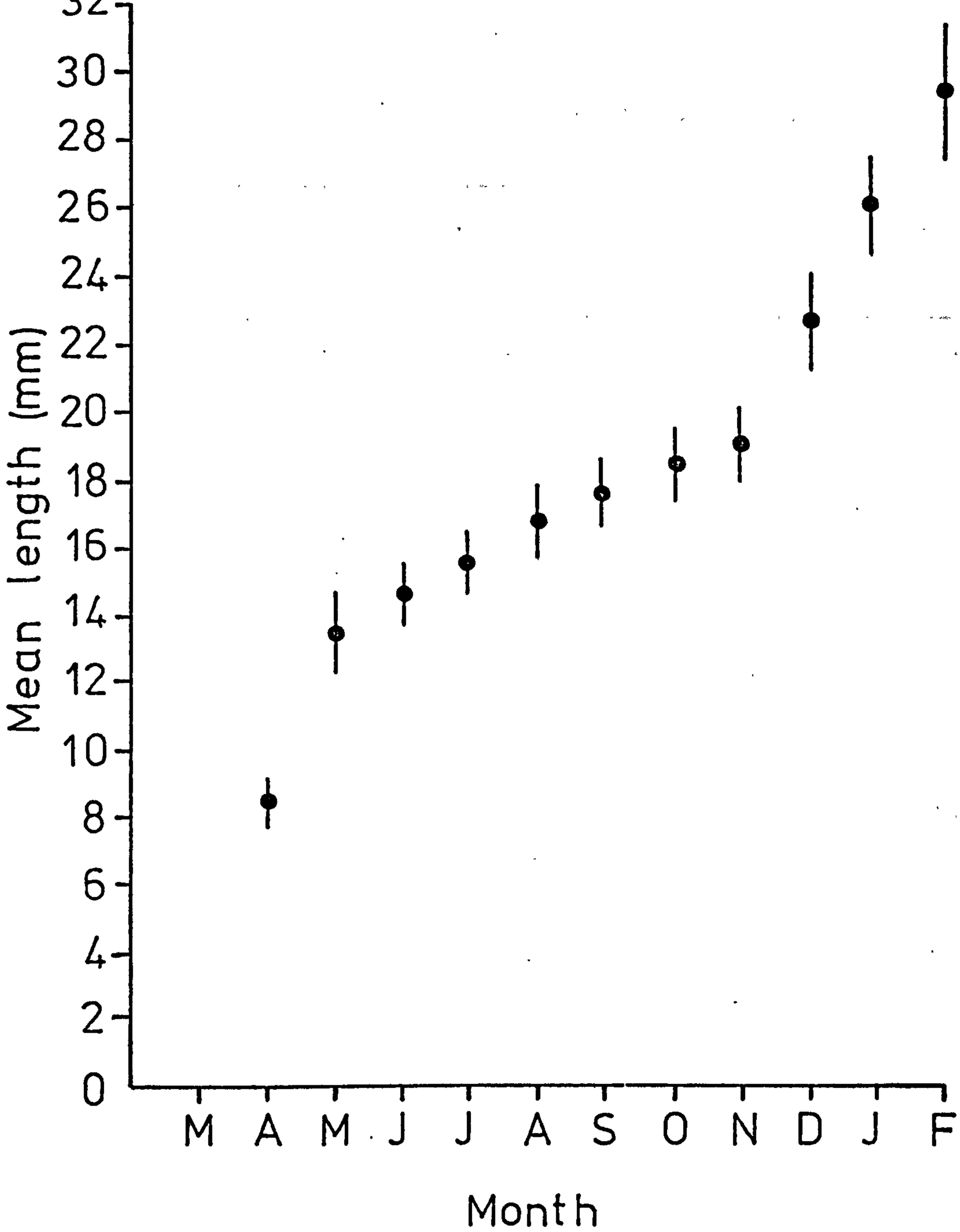

Mean length (mm)
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
M A M J J A S O N D J F
Month

Table 17

Seasonal variation in the degree of infection of G.aculeatus by P.geometra

Month	Number of sticklebacks		Infection	Number of leeches	M.W.B.
	Examined	Infected	(%)		
Aug.	50	1	2.0	1	1.0
Sept.	91	3	3.3	4	1.33
Oct.	119	1	0.84	1	1.0
Nov.	57	0	0.0	0	0.0
Dec.	88	0	0.0	0	0.0
Jan.	33	0	0.0	0	0.0
Feb.	113	3	2.65	3	1.0
Mar.	0	-	-	-	-
April	115	0	0.0	0	0.0
May	102	1	0.98	1	1.0
June	45	0	0.0	0	0.0
July	62	1	1.61	1	1.0

rheotactic response results in movement towards the fish.

Successful attack depends upon the fish species used and its swimming position relative to the searching leeches. Leeches moving towards stone loach and gudgeon on the bottom of the tank attack the fish within 15 minutes. Leeches only attack sticklebacks if the fish are swimming near the bottom of the tank, or if the fish remain stationary for a long enough period for H.marginata to attack from the weed. Attack on sticklebacks generally takes longer than for stone loach and gudgeon, while with goldfish, no successful attack was ever observed. Although leeches react to the presence of goldfish, the searching and attacking movements are usually too slow for infection to occur.

P.geometra react in a similar manner to H.marginata in the presence of a fish. Searching adults move rapidly towards the upper surfaces of stones and weed, whereas young leeches usually swim towards the fish. Attachment to fish then occurs in a similar manner to H.marginata. Attack, however, is much more rapid and can occur within a few minutes upon goldfish or sticklebacks. While the introduction of stone loach and gudgeon initially produces a similar response, P.geometra usually return to a relaxed position unless the fish are swimming continuously. If leeches touch either gudgeon or stone loach when resting on the bottom of the tank, then attack may occur, but it is much slower than for mid-water fish such as sticklebacks or goldfish.

(a.ii.) First attack

The effect of density on the first attack of P.geometra on G.aculeatus was observed at 10°C. Experiments were terminated when at least 66.7 % of the stickleback population had been attacked for the first time at any fixed density of leeches. Results were analysed using the method previously described (Section II. 4). If the ED 50's are plotted against parasite density, a negative exponential curve of the form:-

$$ED\ 50\ (p) = ED\ 50\ (0)\ \exp(-\alpha p) \qquad (14)$$

is obtained. As observed for A.foliaceus, an increase in parasite density decreases the time to first attack but it does so at a constant exponential rate (Figure 32).

(b) Infection pattern

The infection pattern of P.geometra onto fish were analysed by the same method previously described for A.foliaceus (Section III.page 34).

(i) Effect of starvation

The infection pattern of 20 leeches onto one C.carassius at 10°C. was observed over a ten minute period. The mean number of leeches attacking the fish at half minute intervals is indicated in Figure 33 . After ten minutes, a mean number of 7.2 and 10.8 leeches (starved for one and two weeks respectively) had attacked the fish. In both cases, the majority of leeches had attacked the fish after two and a half minutes, but the rate of infection was not constant.

(ii) Effect of host species

The mean number of leeches attacking N.barbatulus and C.carassius at half minute intervals over a ten minute period is indicated in Figure 34 . After ten minutes, a mean number of 2.8 leeches had attacked stone loach and 7.2 leeches had attacked crucian carp. The infection pattern follows a similar model to that previously described (b.i.).

(iii) Effect of substrate

The addition of weed markedly influenced the infection rate of leeches (starved for one week) onto crucian carp. It was observed that a mean number of 7.2 leeches attacked crucian carp after ten minutes without substrate (b.i.). In the presence of substrate, only a mean number of 2.8 attacked after ten minutes (Figure 35). After this, infection occurred quite slowly for the first 50 minutes. Thereafter, the infection

Figure 32

The effect of increasing parasite density on the first attack of P.geometra

Solid line represents the best fit exponential model of the form :-

$$N(t) = x \exp(-yt)$$

where $x = 48.0912$

$y = 0.0827$

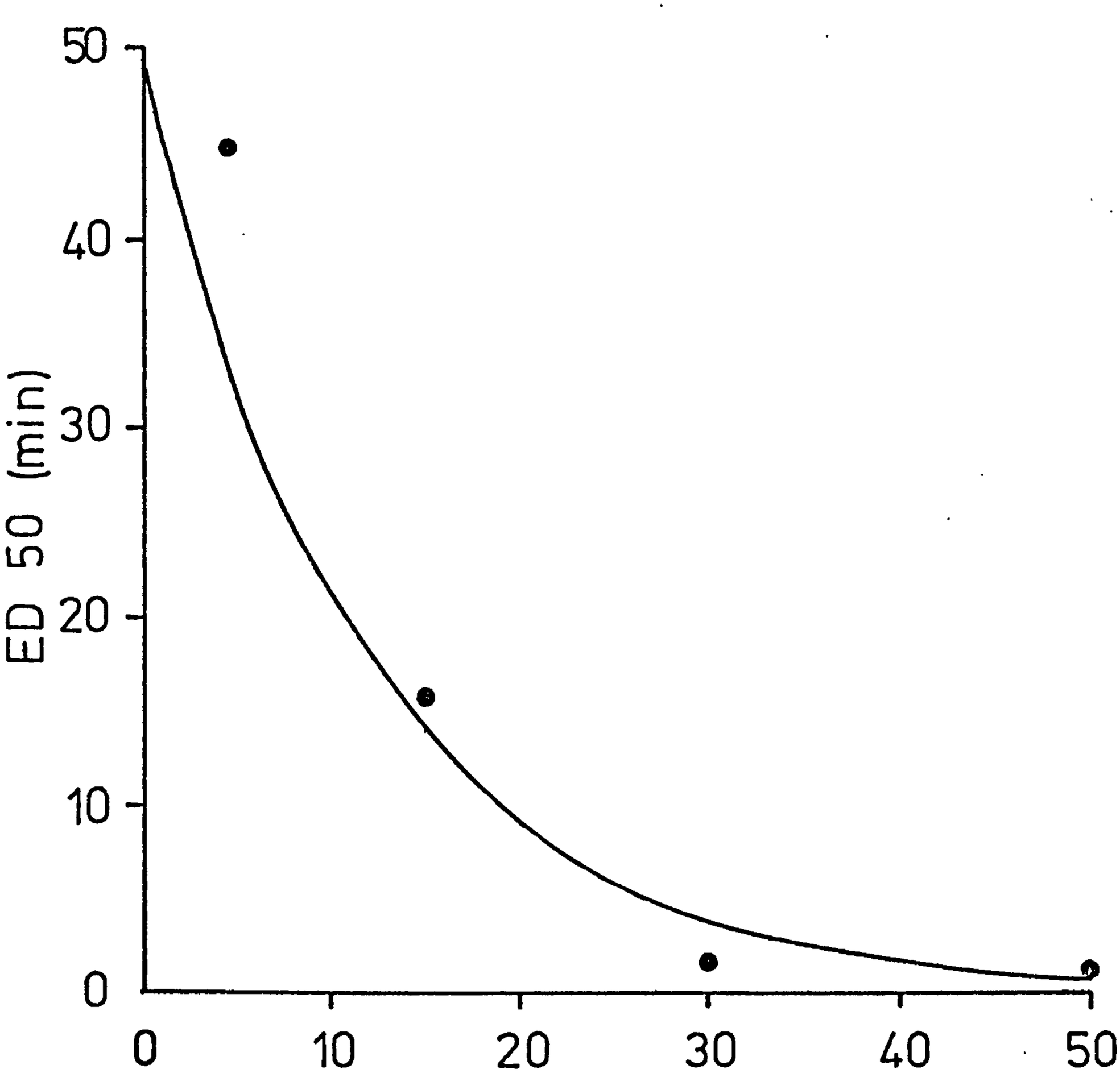

50
40
30
20
10
0
ED 50 (min)
0
10
20
30
40
50
Parasite density

Figure 33

Figure 34

The relationship between the mean number of successful infections per host by leeches on different starvation regimes and the duration of exposure to infection

- One week starvation
- Two week starvation

The relationship between the mean number of successful infections per host by leeches onto different host species and the duration of exposure to infection

- Carassius carassius
- Noemacheilus barbatulus

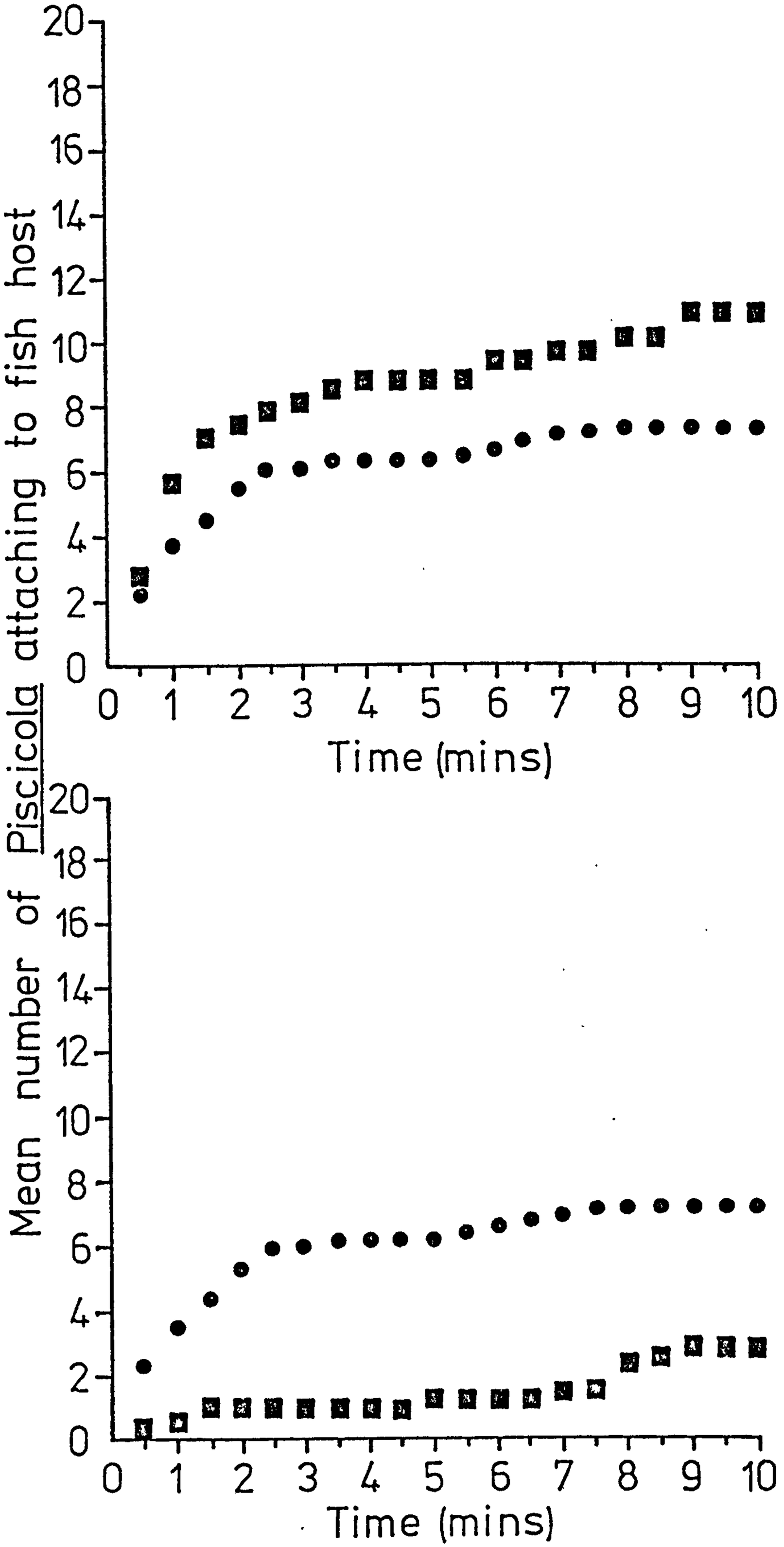
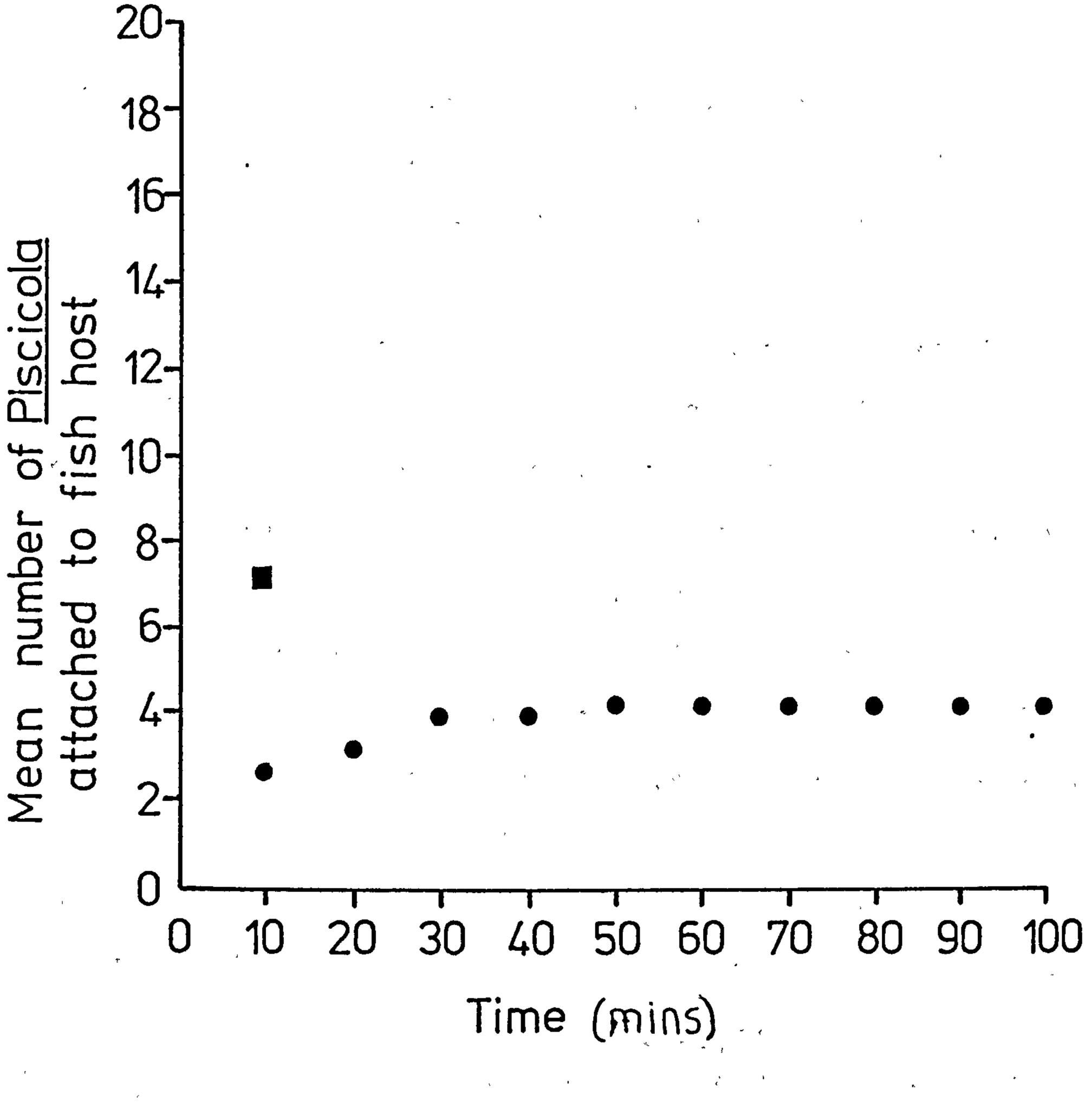

host
0
2
4
6
8
10
12
14
16
18
20
0 1 2 3 4 5 6 7 8 9 10
Time (mins)
Mean number of Piscicola attaching to fish
0
2
4
6
8
10
12
14
16
18
20
0 1 2 3 4 5 6 7 8 9 10
Time (mins)

Figure 35

The relationship between the mean number of successful infections per host by leeches on different substrate regimes and the duration of exposure to infection

■ Without substrate

● With substrate


Mean number of Piscicola attached to fish host
0
2
4
6
8
10
12
14
16
18
20
0
10
20
30
40
50
60
70
80
90
100
Time (mins)

stabilised and after 100 minutes, a mean number of 4.2 leeches had infected the fish.

(c) Feeding and site selection

(i) General observations

H.marginata was observed to feed on the following fish species:- G.gobio, C.carassius, C.gobio, P.phoxinus, N.barbatulus, G.aculeatus and P.pungitius. C.auratus was never attacked by this leech. When feeding on C.gobio, G.aculeatus, P.phoxinus, N.barbatulus and P.pungitius, leeches were observed to feed only on the body region of the fish. On G.gobio and C.carassius feeding occurred on the fins.

Detailed observations were made of H.marginata feeding on gudgeon and sticklebacks. In both fish, a suitable feeding site was found within ten minutes of attack. Blood was discernable in the gut of attacking leeches after half an hour. On gudgeon, feeding was intermittent since violent fin movements often dislodged leeches. The time spent on fish was variable, but never exceeded two days. In cases of H.marginata feeding on sticklebacks, the fish were very weak after several hours and died two-three days later. Leeches caused noticeable damage to the fish in the feeding area with a distinct increase in pigmentation and dermal lesions. Engorged leeches on one stickleback could be eaten by other sticklebacks.

P.geometra was observed to feed on C.auratus, C.carassius, A.anguilla, G.aculeatus, S.erythrophthalmus, C.carpio, P.phoxinus, P.pungitius and N.barbatulus. With the exception of C.auratus, A.anguilla and C.carassius where feeding occasionally occurred in the head region, P.geometra fed on the fins of all other fish species. Although G.gobio was attacked, feeding was never observed and leeches eventually left the fish. Marked variations in feeding and attachment times of leeches were observed on fish. Feeding was intermittent and often affected by fin movements. Noticeable

variations were also observed in the amount of time taken in obtaining blood even by leeches feeding on the same fin (Plates 10 and 11). Copulation sometimes occurred on the fish between feeding periods (Plate 12) and the time spent on the fish often lasted for several days. Feeding on A.anguilla only occurred if the leech attacked in the head region, since the fish usually managed to remove leeches from other areas of its body with its mouth.

(ii) Site selection

Detailed observations were made when 100 P.geometra were exposed to crucian carp at 10°C. After ten minutes, 81% of the leeches had attached to the fish. Initial attachment of the leeches (Plate 13) (as determined by the position of the posterior sucker) shows a slight preference for the anterior and middle sections of the body and for the pelvic and pectoral fins (Table 18). There is no distinction between attachment to the left or right sides of the body and fins (χ^2= 0.6533, not significant at P>0.05). χ^2 analysis indicates a significantly greater number attached to the body as opposed to the fins (χ^2= 6.5308 at P>0.05). Once the leech is attached to the fish, its anterior sucker is used to find a suitable feeding site on the fish. After ten minutes, 65% of the leeches were in the process of feeding with site preferences for the pelvic, pectoral and caudal fins. There is no distinction between attachment to the left or right sides of the body and fins (χ^2= 0.8033 at P>0.05). There are a significantly greater number of leeches feeding on the fins as opposed to the body (χ^2= 14.7846 at P>0.05). When feeding on the fins, the anterior sucker is attached to the edge of the fin (Plates 14 and 15), whereas the posterior sucker can be in any position on the fish depending on the initial site of attachment. Feeding on the anterior body occurs in the head region in areas devoid of scales. In the middle and posterior regions

Table 18

Site distribution of P.geometra on C.carassius

Site	Total	Position		Chi-square	Significance
		Left	Right		
Anterior sucker					
Fins	48	26	22	0.3333	n/s
Caudal	9	2	7	2.7778	n/s
Pectoral	13	8	5	0.6923	n/s
Dorsal	5	2	3	0.2000	n/s
Anal	5	3	2	0.2000	n/s
Pelvic	16	11	5	2.2500	n/s
Body	13	8	5	0.6923	n/s
Anterior	7	5	2	1.2857	n/s
Middle	3	2	1	0.3333	n/s
Posterior	3	1	2	0.3333	n/s
Ventral	3	-	-	-	-
Dorsal	1	-	-	-	-
Total	65				
Posterior sucker					
Fins	29	18	11	1.6897	n/s
Caudal	6	2	4	0.6667	n/s
Pectoral	8	5	3	0.5000	n/s
Dorsal	0	-	-	-	-
Anal	4	3	1	1.0000	-
Pelvic	11	8	3	2.2727	n/s
Body	46	23	23	0.0000	n/s
Anterior	24	12	12	0.0000	n/s
Middle	15	9	6	0.6000	n/s
Posterior	7	2	5	1.2857	n/s
Ventral	4	-	-	-	-
Dorsal	2	-	-	-	-
Total	81				

Table 19

Site distribution of P.geometra on C.carassius

	Percentage of the original number on the fish							
	Fins					Body		
Post.sucker / Time	Caudal	Pectoral	Dorsal	Anal	Pelvic	Ant.	Mid.	Post.
10(mins)	7.41	9.88	0.0	4.94	13.58	37.04	18.52	8.64
20(hrs)	4.94	4.94	0.0	3.70	7.41	14.81	12.35	3.70
44(hrs)	3.70	3.70	0.0	2.47	0.0	4.94	7.41	1.23
68(hrs)	2.47	2.47	0.0	1.23	2.47	1.23	0.0	0.0
92(hrs)	0.0	2.47	0.0	1.23	0.0	0.0	0.0	0.0
116(hrs)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ant.sucker								
10(mins)	11.11	16.05	6.17	6.17	19.75	13.58	3.70	3.70
20(hrs)	11.11	3.70	7.41	4.94	8.64	3.70	6.17	0.0
44(hrs)	4.94	3.70	3.70	3.70	0.0	1.23	3.70	0.0
68(hrs)	2.47	2.47	1.23	0.0	0.0	0.0	0.0	0.0
92(hrs)	0.0	0.0	0.0	0.0	2.47	0.0	0.0	0.0
116(hrs)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

feeding occurs at the junction of the fins with the body. This pattern of site selection remains fairly constant throughout the length of time spent on the fish (Table 19). Feeding occurs at intermittent periods, the duration of which are partly dependent on the number of leeches feeding and on the fish reaction to infection.

(d) Emigration

(i) General observations

On completion of feeding, both species of leech leave the fish host and digest their meal. In H.marginata, the anterior sucker attaches to the substrate, the posterior sucker then moves into a position immediately behind the anterior sucker and a form of looping movement occurs until a suitable resting site is found. This is usually under stones, or completely flat on the tank walls. Leeches were not observed to move from these positions during digestion of the blood meal. The length of time for digestion varies in individual leeches and in many, the response to feed on fish does not recur for periods of up to six months.

P.geometra leaves the fish in a similar manner to that of H.marginata. However, if the fish was in mid-water, both suckers were released and the leech dropped or swam to the substrate. In a relaxed position, both suckers were firmly attached to the substrate. Digestion at 20°C. may take place in up to several weeks, but it is dependent on the size of the leech and the amount of blood taken during the previous meal.

(ii) Experimental results

The attachment of P.geometra to C.carassius at 10°C. is shown in Figure 36 . These results were obtained from the experiment on changes in site selection (Section II.3e.ii and iii). Detachment decreased rapidly with time and after 116 hours, all leeches had left the fish. The relationship between the mean proportion of leeches on the fish (N) and the

Figure 36

The mean proportion of the number of

<u>P.geometra</u> emigrating from the fish

host over a given time period

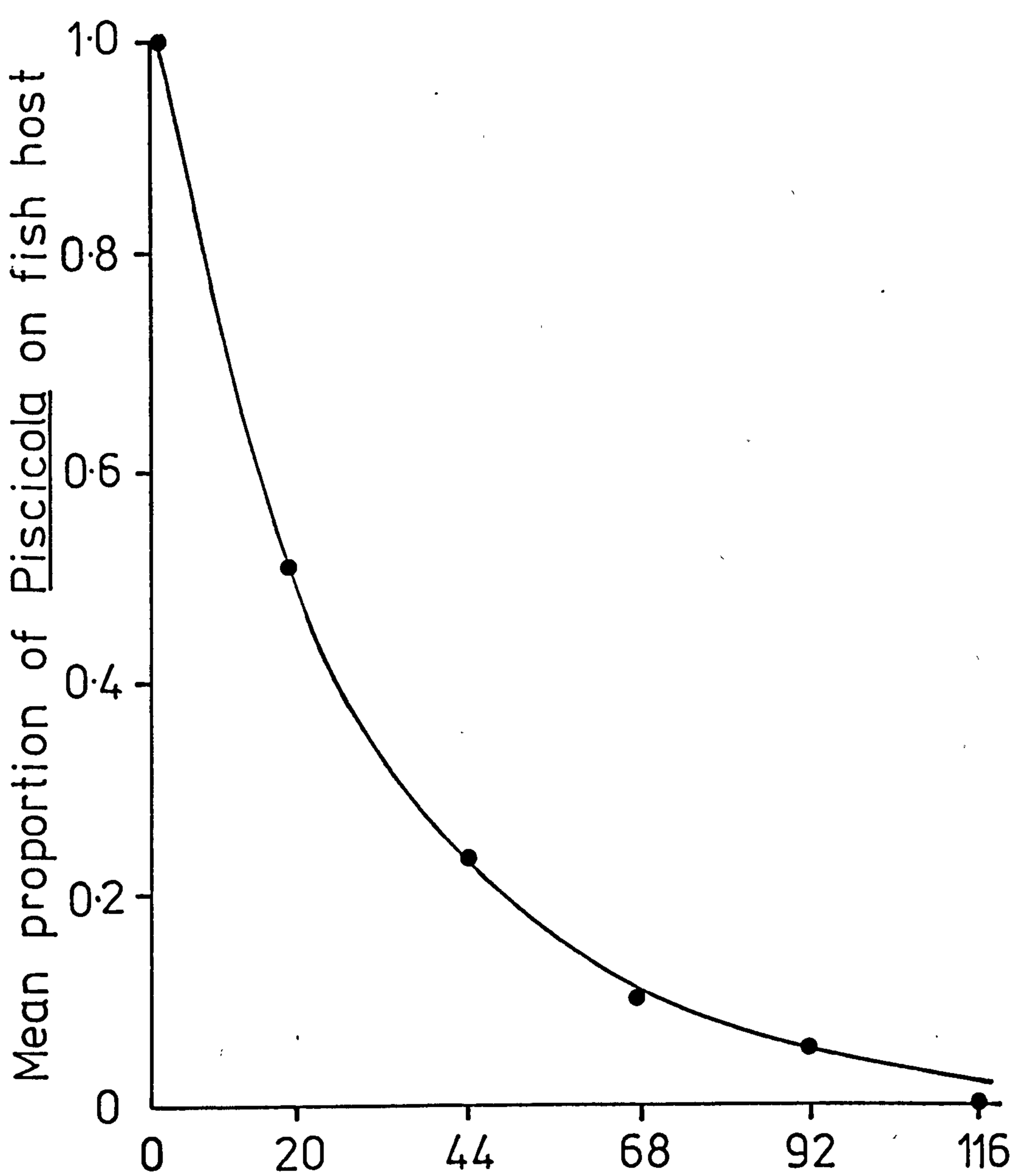
Solid line represents the best fit

exponential model of the form:-

$$N(t) = x \exp(-yt)$$

where $x = 0.9989$

$y = 0.0331$


1·0
0·8
0·6
0·4
0·2
0
Piscicola on fish host
Mean proportion of E
0
20
44
68
92
116
Time (hours)

time (t) can be described by an exponential model of the form:-

$$Nt = x \exp(-yt)$$

where y represents the emigration rate.

The emigration rate at different leech densities shows a constant pattern (Figure 37) and can similarly be described by the equation above. As density increases, the length of time spent on the fish also increases and the emigration rate decreases. A plot of y (emigration rate) against parasite density indicates a negatively linear relationship (Figure 38). This suggests that the emigration rate from the fish is influenced by density dependent factors. As the number of leeches increases, competition for feeding sites occurs.

(e) Growth

At 20°C., P.geometra requires eight feeds before the first cocoons are laid after 22 days (Figure 39). Between feeds, growth is about 2 mm. in length per leech but the time required between feeds increases (Figure 40).

(f) Survival

Under the experimental regime at a temperature of 10°C., recently hatched leeches have a maximum life span of approximately 30 days, with a mean survival time of 21 days. Figure 41 , represents the proportion surviving (Nt) against time (t) as a function of the initial number isolated (30). If the instantaneous death rate were constant, then an exponential decline in population size would be expected (Anderson and Whitfield, 1975). However, the results indicate that the instantaneous death rate increases with time as a function of age.

The effect of starvation on the survival of adult leeches at 10°C. and 20°C. (Figure 42) indicates a maximum life span of 13 weeks with a mean survival rate of 7 weeks and a maximum life span of 5 weeks with a mean life

Figure 37

The mean proportion of the number of different densities of P.geometra emigrating from the fish host over a given time period

Solid lines represent the best fit exponential model of the form:-

$$N(t) = x \exp(-yt)$$

where at Parasite density

2	●	x = 0.9442 y = 0.5522
5	Δ	x = 0.8465 y = 0.4704
10	O	x = 0.8876 y = 0.4011
20	■	x = 1.3694 y = 0.1816

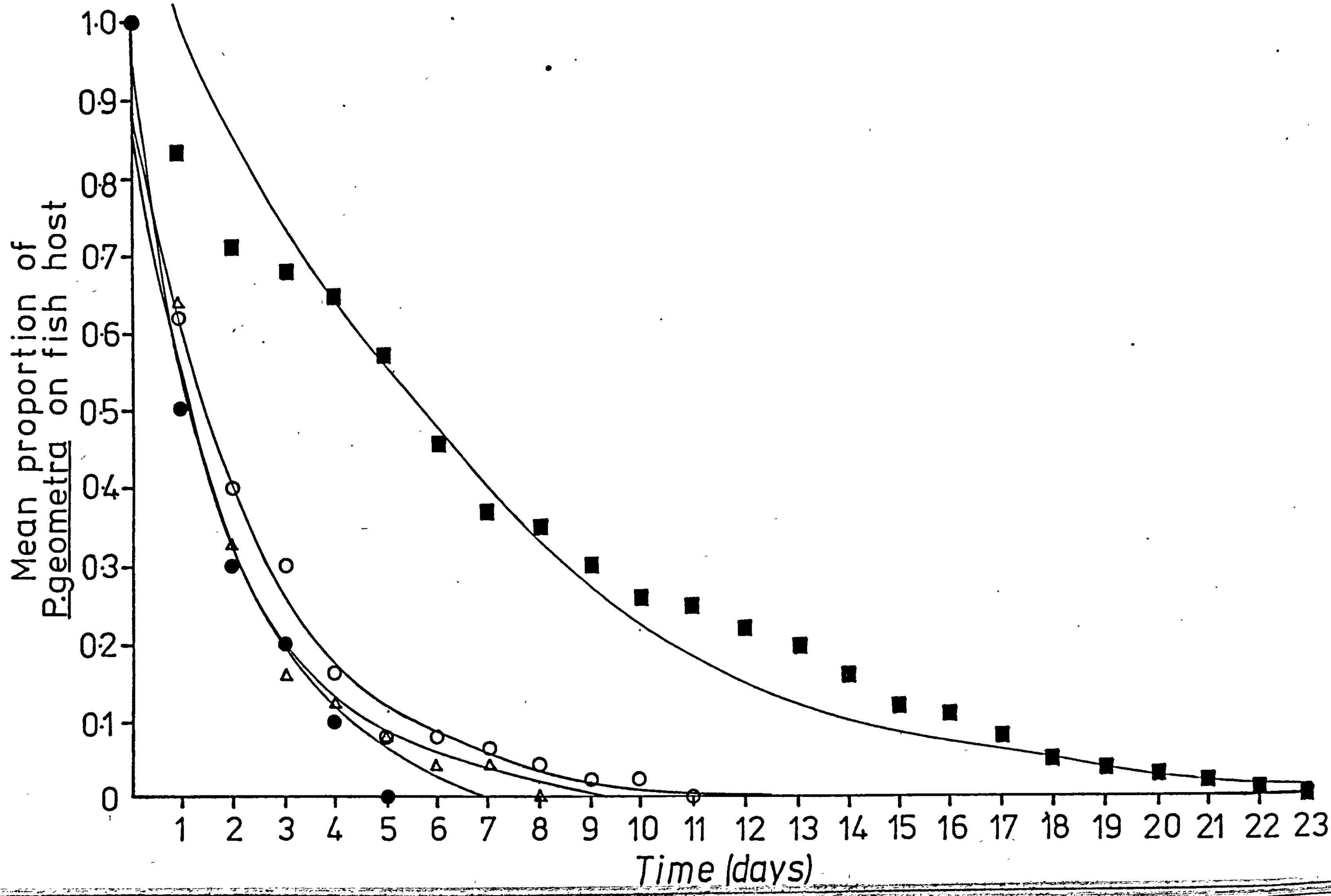

Mean proportion of P.geometra on fish host
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Time (days)

Figure 38

Relationship between the emigration rate (y) of P.geometra and increasing parasite density

Linear equation:-

$$y = 0.5870 - 0.0201x$$

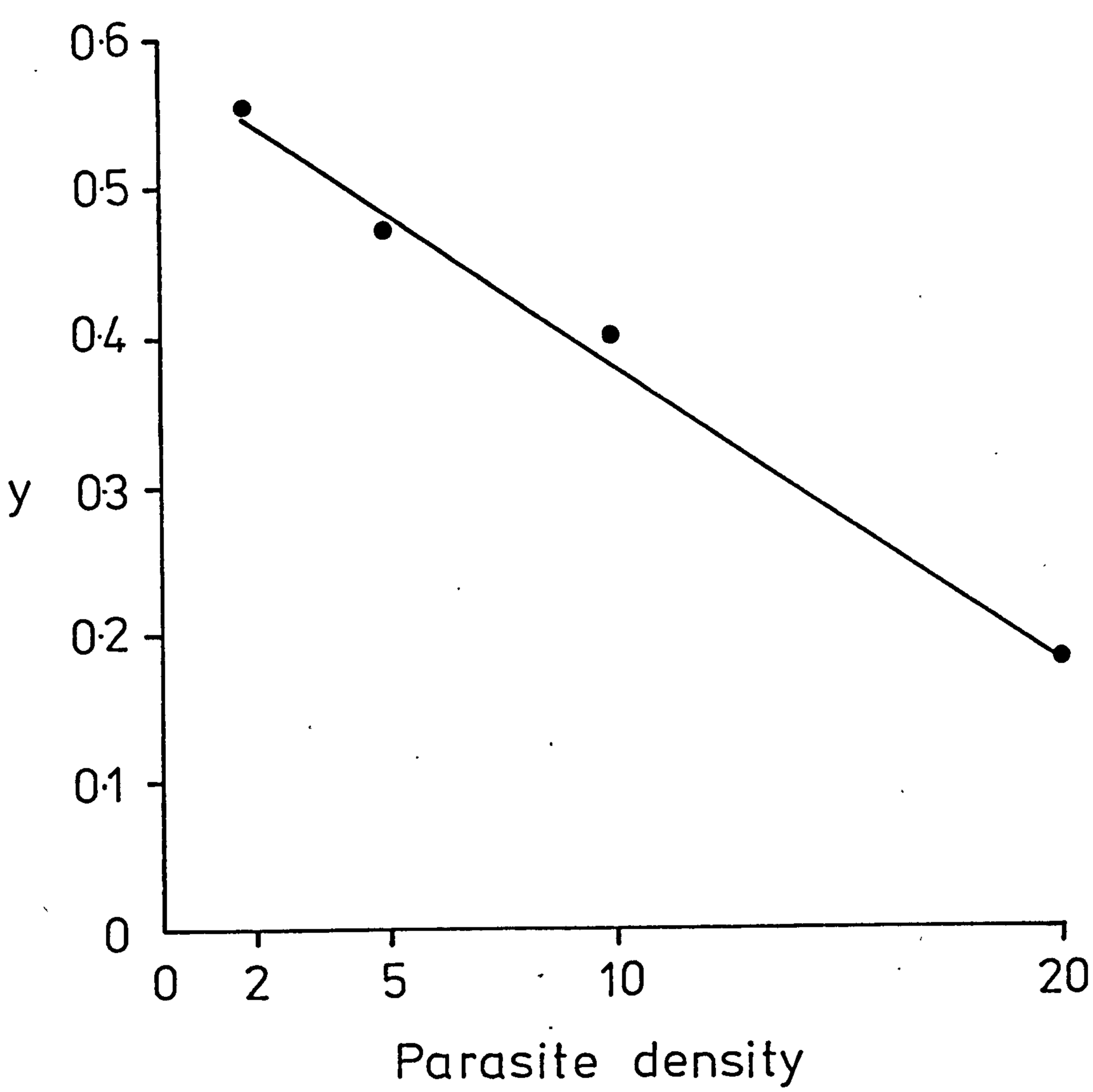

0·6
0·5
0·4
y 0·3
0·2
0·1
0
0
2
5
10
20
Parasite density

Figure 39

Number of feeds required by P.geometra to reach maturity (i.e. when first cocoons are laid) at 20° C.

● = Observed means. Vertical bars represent 95% confidence limits.

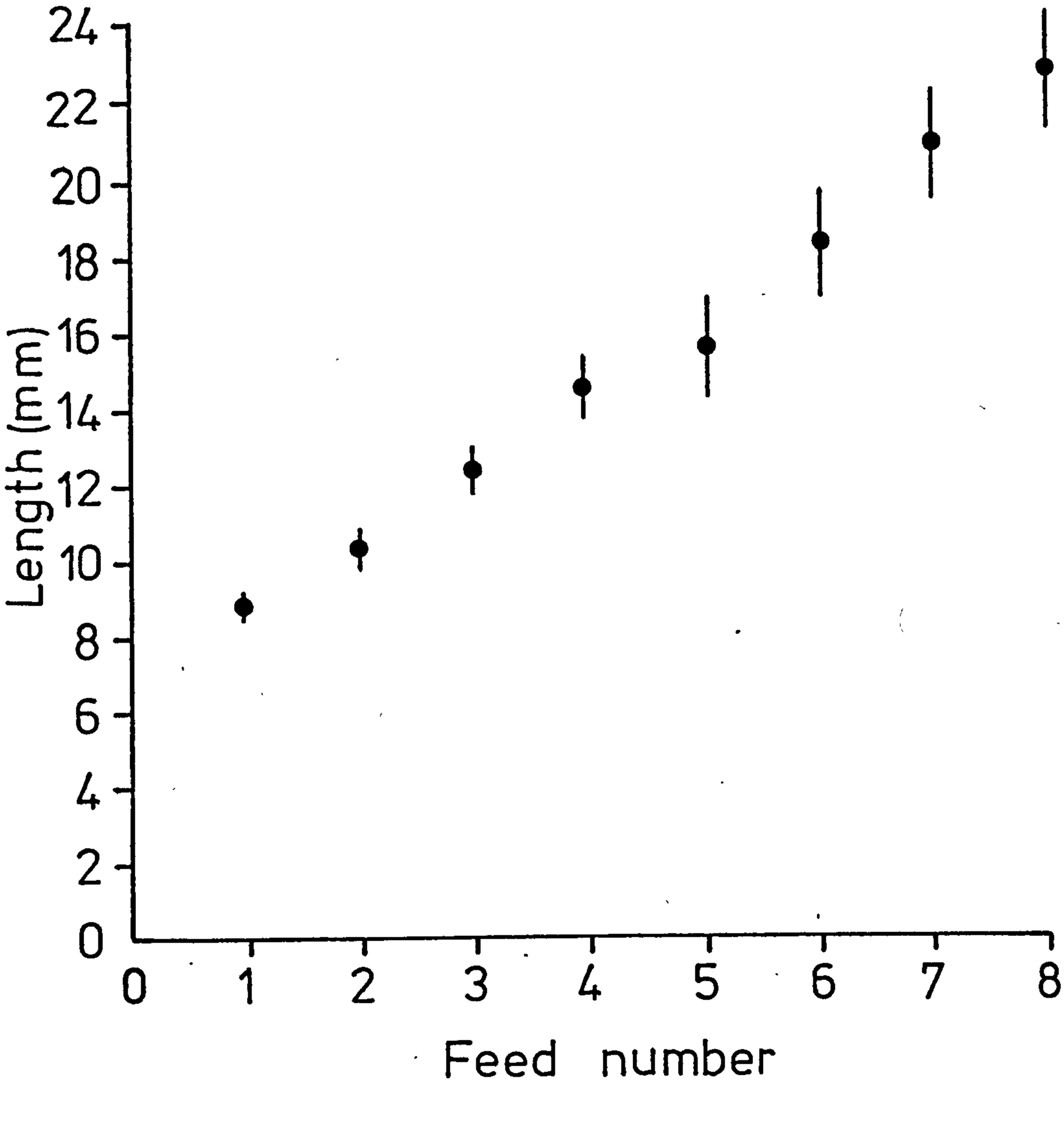

Length (mm)
0
2
4
6
8
10
12
14
16
18
20
22
24
0
1
2
3
4
5
6
7
8
Feed number

Figure 40

Growth of P.geometra at 20° C.

● = Observed means. Vertical bars represent 95% confidence limits.

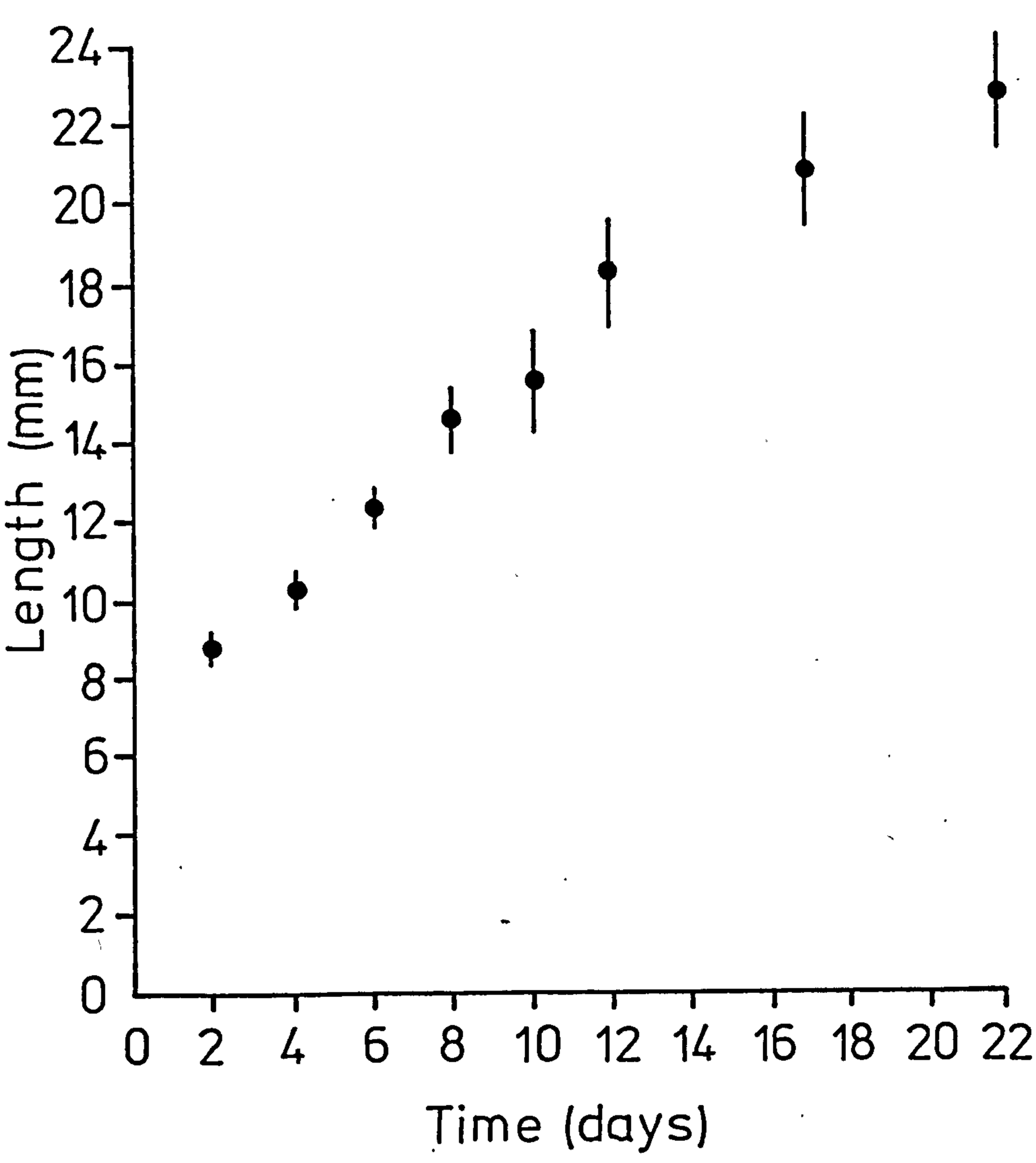

Length (mm)
0
2
4
6
8
10
12
14
16
18
20
22
24
0
2
4
6
8
10
12
14
16
18
20
22
Time (days)

Figure 41

The observed number of young P.geometra surviving at daily intervals, shown as the mean proportion (Nt) of the initial population size.

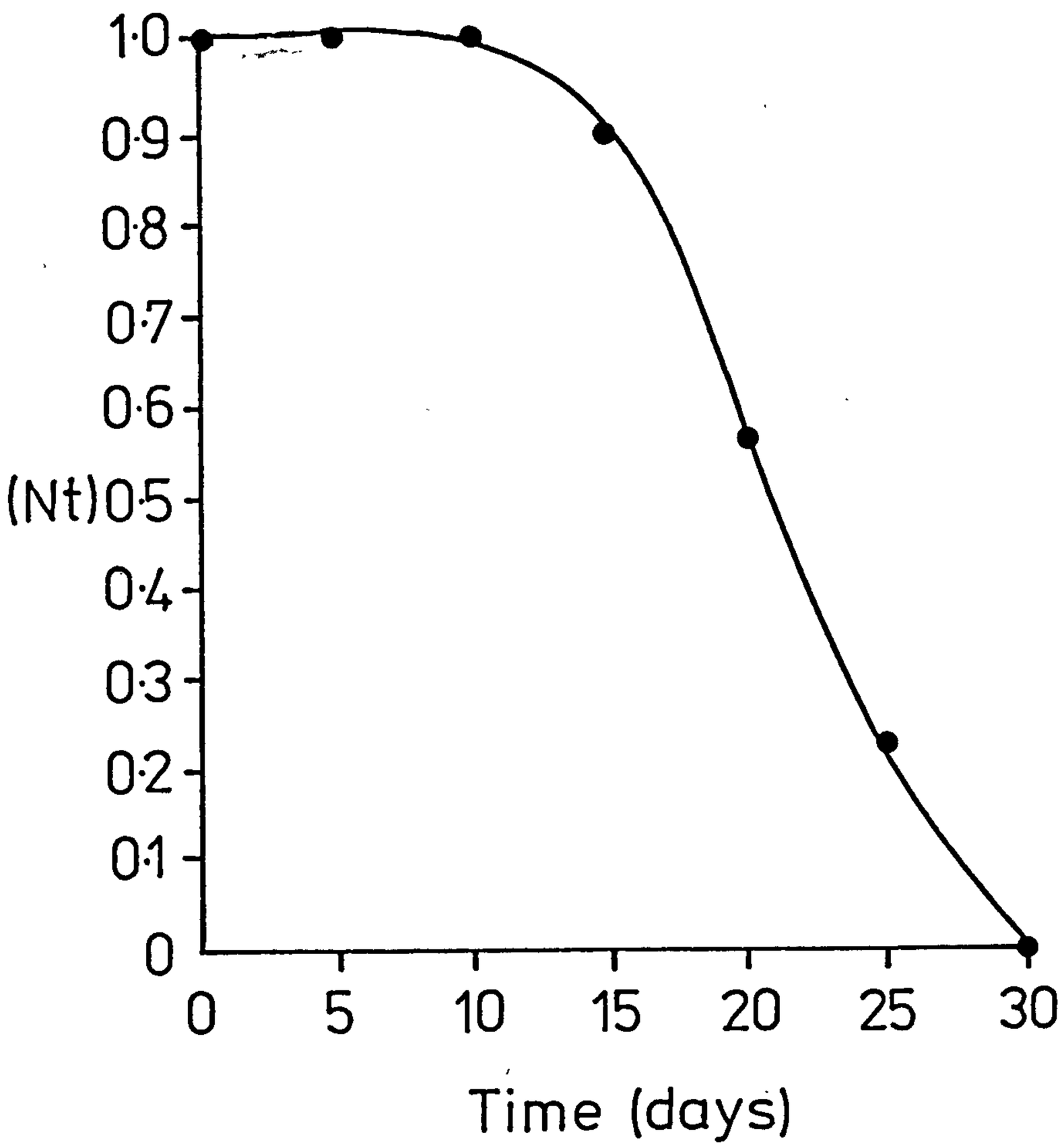
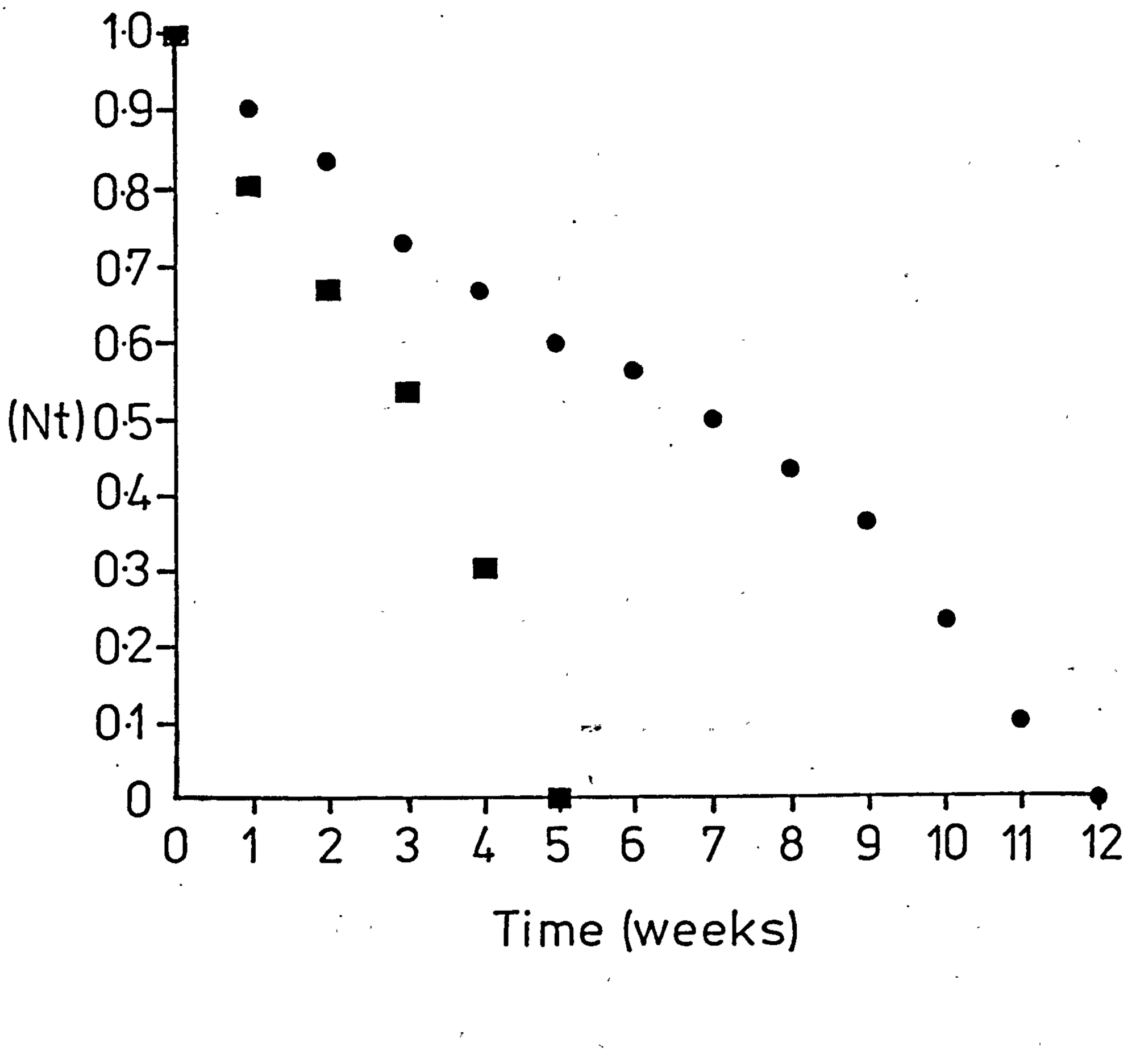

1·0
0·9
0·8
0·7
0·6
(Nt) 0·5
0·4
0·3
0·2
0·1
0
0
5
10
15
20
25
30
Time (days)

Figure 42

The observed number of adult P.geometra surviving at weekly intervals at different temperatures, shown as the mean proportion (Nt) of the initial population size.

■ 20° C.

● 10° C.


1·0
0·9
0·8
0·7
0·6
(Nt) 0·5
0·4
0·3
0·2
0·1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (weeks)

span of 3.2 weeks respectively.

No fed leeches in stock were ever observed to survive longer than one year at either temperature.

Discussion

Mann (1964) and Terekhov (1967) suggested that the life cycle of P.geometra lasts for less than one year. This is supported by present observations. Although the natural parasitic phase was recorded upon sticklebacks, the low incidence of P.geometra is surprising. Terekhov showed that in April, leeches from 22-40 mm. in length predominate, while at the end of May, only leeches from 4-24 mm. in length were present. In the summer, leeches in the length range 25-40 mm. were only present occasionally. He therefore went onto suggest that in spring, mass mortality of adults occurs, connected with the end of the life cycle. Terekhov confirmed these conclusions on the survival of leeches in the laboratory where leeches did not live longer than nine months.

If the stucture of the free living population of P.geometra present at Lea Bridge is also representative of the parasitic phase, then Terekhov's observations are supported. Thus, in February, overwintered adult leeches begin to reproduce and deposit cocoons, presumably associated with an increase in water temperature or day length. Adult mortality occurs in March and the cocoons, which take several months to develop at low temperatures, hatch in April. While the present study suggests that mortality amongst the adult population may occur in February, the studies of Terekhov (1967) and Halvorsen (1972) suggest that mortality occurs in April-May. This may be associated with an earlier increase in temperature and warmer waters in this country than those found in Russia (Terekhov, 1967) and in Norway (Halvorsen, 1972).

Growth of P.geometra is fairly rapid in the first month until maturity is reached in May at approximately 11-15 mm. length. The newly mature adults begin to reproduce and cocoons are laid from May until November. With the relatively higher summer temperatures, cocoons hatch within 13 - 20 days (Terekhov, 1966) and a new generation is likely to appear at monthly intervals until November when cocoon production stops. Present field and laboratory studies suggest that the lower rate of growth during the summer is associated with the production of cocoons and thereafter in the winter months a higher growth rate is apparent until the leeches die. In the laboratory, if leeches are allowed to feed when hungry, then the growth rate at 20°C. is approximately one millimetre/day and maturity is reached within three weeks after eight feeds. New generations of leeches can occur within four to five weeks which supports field observations. The growth rate is likely to be a function of the temperature, number of feeds and the time spent between feeding.

This situation may apply to leeches hatched in the spring and early summer, but it does not explain the apparant disappearance of leeches hatched in the late summer and autumn. Without data for the occurrence of leeches on the fish population, or on alternative substrates inhabited as a result of plant reduction in the winter, it is impossible to draw further conclusions.

Bauer (1958a) investigated the seasonal cycle of P.geometra (see Section 1V.2. page 61).If this situation occurred in the present survey, then leeches in the 15 - 29 mm. length group in May, might consist partly of overwintering young leeches(which have a low growth rate in winter temperatures) having left the fish as well as the new generations of leeches hatched in April. It is considered that a detailed survey of the fish population is required before some of the present anomalies can be explained.

The life span of recently hatched P.geometra at 10°C., lasts for a maximum of 30 days unless feeding occurs. This is relatively long in comparison with A.foliaceus and may largely be a function of their lower activity. Young P.geometra spend most of the time in a relaxed position on stones and weed. Activity is only increased in the presence of a fish host, when leeches respond either by swimming towards the fish or assuming a vertical position. This reaction therefore minimises the energy expenditure of leeches and maximises the length of time available for successful infection to occur.

In hungry leeches, the start of the searching and attacking phase is initially provoked by currents caused by fish swimming movements. The variation in response by P.geometra and H.marginata is largely a function of the swimming behaviour of different fish species. Searching by the latter is slower than that of the former and attack is restricted to bottom swimming fish such as N.barbatulus and G.gobio. P.geometra is usually limited to mid-water species such as G.aculeatus, C.auratus and C.carassius. Although Hemiclepsis attack mid-water species and P.geometra bottom living species, the relative response is generally slower in both cases. It is suggested that in a situation where a choice of species are available, that the faster response evoked by a suitable fish will determine which species is preferentially attacked. This is verified by the infection of P.geometra on C.carassius and N.barbatulus, where faster responses to the former produces a higher infection rate.

In P.geometra, the initial attacking response appears to be unaffected by parasite density. Under experimental conditions where substrate is available, P.geometra occurs principally on weed. General observation indicates that the leeches are apparently randomly distributed on the plants. This segregates the contact range of individual leeches. Occasionally,

leeches may contact each other when reacting to fish. This does not appear to affect the searching efficiency of the parasites. As in the case of A.foliaceus, the infection rates of P.geometra are largely influenced by heterogeneity amongst the leech population. This is presumably a reflection of nutritional status since increased starvation time produces higher levels of infection.

The experimental results suggest that the presence of substrate induces a decline in infection, but under natural conditions this correlation may not be so direct. The behavioural response of fish to leech infection is generally one of increased swimming activity. This brings the fish into increasing contact with searching leeches. While the addition of substrate provides a greater surface area from which leeches may attack, it also provides a refuge for the fish. Thus, after initial leech contact, the leech response declines due to decreased behavioural activity of the fish. Under natural conditions it is suggested that the absence of plants (during the winter months) will restrict leeches to bottom substrates and will therefore decrease the chances of attack occurring. Also the fish response maybe to swim upwards away from leech contact. However, since P.geometra is non-specific, attacks may therefore be restricted to bottom dwelling fish during the winter months. In the spring, with an increase in vegetation, greater infections may occur due to:- a) larger numbers of fish attracted to the substrate in relation to their feeding and behavioural activities b) greater diversity of fish species in relation to the distribution of leeches on different substrates and depths of water.

In winter, the reduced chance of feeding is compensated in P.geometra by the long term survival strategy. The slow digestion rate of blood meals at low temperatures enhances the survival of the leech during the winter. At 10°C., P.geometra can survive for a maximum starvation period of up to three months.

In P.geometra, the initial phase of attachment onto C.carassius is dependent upon the areas on the fish with which the leeches first come into contact and which also provide a greater surface area. Thus, as the fish swims forward, leeches attach to the anterior and middle regions of the body and the pectoral and pelvic fins, with relatively less attachment occurring on the posterior region of the body, anal, dorsal and caudal fins.

Once attack is complete, both leech species normally find a feeding site within fifteen minutes. The area chosen for feeding is largely dependent upon the prevalence of scales. In fish which have no scales, or very small scales, such as P.phoxinus, N.barbatulus, G.aculeatus, C.gobio and P.pungitius, feeding in H.marginata occurs on the body. In fish which have fairly large scales, such as C.carassius and G.gobio, feeding occurs on the fins. In all 12 fish species observed, P.geometra fed on the fins, with occasional feeding in the head region of C.carassius, C.auratus and A.anguilla. In both fish leeches, preferable feeding sites therefore seem to be restricted to areas which are easily accessible to blood, thus, scaleless areas or fins are selected.

Indirect evidence for the feeding preferences of H.marginata for bottom dwelling fish under natural conditions is provided by the work of Letch (1977) and Letch and Ball (1979). They investigated the distribution of trypanosomes in ten different freshwater fish species and suggested that they should all be classified as one species, namely Trypanosoma cobitis. Certainly, if H.marginata is indiscriminate in the choice of fish hosts, especially between feeds, then the transmission of only one species of trypanosome to a number of different host species is a realistic possibility. The incidence of T.cobitis in the area they studied maybe indicative of the feeding patterns of H.marginata, since it was the only leech vector found. Bottom living fish such as N.barbatulus, G.gobio and C.gobio show a higher

incidence of trypanosome infection (50 - 71%) than midwater dwelling species such as P.phoxinus, G.aculeatus and P.pungitius (6 - 18%). Fish with fairly large scales such as L.cephalus, R.rutilus and P.fluviatilis showed no infection with T.cobitis. This again indicates the preference of H.marginata for bottom dwelling, scaleless fish.

At a basic level, the overall feeding strategy of these fish leeches is largely influenced by the initial attacking response to various fish species and the selected feeding sites on different fish species. If the parasitic phase is one of feeding in the shortest possible time, then factors which extend the length of time spent on the fish will adversely affect the basic feeding strategy. In general, the operation of density-dependent factors upon many fish/host parasite systems maybe attributed to two basic mechanisms, namely intraspecific competition for finite resources and immunological responses by the fish host. In the first case, intra-specific competition amongst individual parasites on the host should lead either to a decrease in parasite reproduction or to an increase in parasite mortality (Mills, Anderson and Whitfield, 1979). At high densities of P.geometra it is possible that both density-dependent factors are operating. With such densities, competition for feeding sites leads to an increase in time required for completion of feeding. This increases the length of time spent on the fish. The presence of a host immunological response was not investigated in the present work. However, two types of behavioural response were observed. Fish heavily infected with leeches often "jump" out of the water. This dislodges leeches. In addition, increased fin movements prevent the leeches feeding. Feeding may also be influenced by the amount of decreased blood available when large numbers of leeches are feeding at any one particular feeding site. This may explain why individual leeches at any one site show differential rates in the amount of time required for

completion of feeding.

Increases in the amount of time spent feeding in relation to parasite density maybe analagous to the effects of interference observed in many predator/prey and host/parasitoid situations. It might be expected that competition at high densities will increase emigration, since it is more profitable for leeches to select lightly infected hosts. In the experimental densities used, however, leeches did not leave the fish until feeding was complete. Regardless of density, the basic strategy is still to complete the feeding process once a host has been attacked. Even so, the increased time spent on the fish maybe more beneficial in energy and time cost than the possible loss in time and energy which might be used in searching out new hosts. Presumably at very high densities where parasite induced host mortality occurs then this strategy breaks down.

It has been mentioned that records of seasonal changes in the occurrence of P.geometra are confusing and that some workers suggest that the leech does not leave the fish after feeding (Needham, 1970; Canning et al. 1973). This situation might be explained by considering the host/parasite association at high levels of infection. Theoretically, if recruitment, parasitism and emigration by individual leeches continues throughout the year, then when high densities of leeches are available (during the spring and summer), the increased time spent on the fish (due to competition) may suggest permanent infection.

V. Anodonta cygnea (L.)

Introduction

The British freshwater bivalves contain three major families, namely the Unionidae (mussels), the Dreissensiidae (zebra mussels) and the Sphaeridae (pill and orb shells). With the exception of one species, Dreissena polymorpha (Pall.) (which has a free swimming veliger larva), the freshwater bivalves incubate their eggs and young. In the Sphaeridae, larvae develop within the gills of the adult and are then released for a free living existence. By comparison, the Unionidae possess a larval stage (glochidium) which is highly adapted for a parasitic life upon fish.

Three genera of Unionidae occur in Britain and their distribution has been analysed by a number of workers (Haas, 1910; Boycott, 1936; Ellis, 1947, 1951; Negus, 1966). The present study is concerned with glochidia larvae of Anodonta cygnea Linnaeus, 1758.

Observations on the reproductive biology of Anodonta sp. have largely been concerned with the seasonal availability and production of glochidia by mature adults (Harms, 1907a, 1907b, 1909; Lefevre and Curtis, 1910; Coker et al., 1921; Bloomer, 1946; Negus,1966; Wood, 1974a). These workers indicated that although mature glochidia were present in the gills of the parent mussel in October, actual release of larvae (initiated by an increase in water temperature) did not occur until the following spring. As pointed out by Guisti et al. (1975), these workers only considered the development of glochidia in the adult mussels, little attention being paid to seasonal variations in the incidence of glochidia in fish populations. Guisti et al. (1975) suggested that the elimination of glochidia is related to a decrease in temperature and they showed that glochidia of A.cygnea are present on fish from early November until late April. The presence of a seasonal cycle in incidence of

glochidia on their fish hosts was recently confirmed in Britain (Campbell, 1974; Dartnall and Walkey, 1979). Although the parasitic phase is seasonal, the infection of glochidia on different host species maybe related to variations in fish behaviour and activity during the year. Guisti et al. (1975) noted that although Lepomis gibbosus and Perca fluviatilis were parasitized in December, Tinca tinca were not infected until January. Peak infection was recorded in P.fluviatilis in February but not until April in T.tinca.

Adult A.cygnea produce enormous numbers of glochidia with up to several thousand being released by one adult (Harangy, Balazs and Berg, 1964, quoted by Lukacsovics and Labos, 1965). Glochidia are discharged through the exhalent aperture of the adult mollusc and are connected together by muc us threads. In still water, the glochidia sink to the bottom, but in currents, the connective byssus thread may act as a "dragline" and increases the time that the larva spends afloat (Wood, 1974b). The byssus may also act as an attachment organ. Glochidia are immobile and dependent chiefly upon water currents for transfer (Lefevre and Curtis, 1910; Wood, 1974b).

Initial contact with fish is dependent upon the respiratory currents created by the fish (Paling, 1968) and by the behavioural activity of fish during feeding and spawning (Guisti et al., 1975). The latter demonstrated the site distribution of glochidia on five species of fish and found that in L.gibbosus, T.tinca and Atherina boyeri (L.), the gills were preferential sites, while the pectoral fins in P.fluviatilis and the ventral fins in Esox lucius were preferred. They suggested that the observed distributions were related to the different behavioural activities of fish species. The feeding behaviour of potential hosts may often bring fish into close proximity of aggregations of glochidia

settled on the bottom substrate. Consequently, it is more likely that the lower region (particularly the head region) of the fish will become infected. Wootten (1973) recorded preferential sites for the gills in brown trout, rainbow trout, perch, ruffe and roach, while in sticklebacks, the fins were selected. High infections of glochidia upon the fins of sticklebacks have also been demonstrated by Dartnall and Walkey (1979).

Following initial contact with the host, permanent attachment depends upon the behaviour of the glochidium. Chemosensitive hairs inside the valves of the glochidium are stimulated by proteins in the mucous of the fish epidermis, producing contraction of the adductor muscles and the valves shut (Lukacsovics and Labos, 1965; Wood, 1974b). Permanent contraction relies upon the continued production of mucous. Within several hours, the glochidium becomes enclosed in a cyst caused by a proliferation of cells in that area (Arey, 1932a). The length of time taken to develop on the host may depend on a number of factors, such as temperature, species and condition of host and age of glochidia (Howard and Anson, 1922). When metamorphosis is complete, extrication from the host tissue occurs and the newly formed adult drops to the substrate and continues with a free living existence.

Several workers (Reuling, 1919, Arey, 1932b) have noted that reinfection of fish previously exposed to glochidia can cause localized tissue responses. A cyst is produced around the larva but after several days it is sloughed off. Metamorphosis is not completed.

Heavy infections of glochidia amongst fish populations are known. However, some fish species are more susceptible to glochidiosis than others. Meyers and Milleman (1977) exposed six species of salmonid fish to varying densities of glochidia of Margaritifera margaritifera (L.).

Several factors were noticeable. Infection is related to the density of exposure and to host species. Increasing the density of glochidia exposed to fish did not produce a proportional increase in the number of glochidia attached. Similarly, some species are more susceptible to attack than others and mortality is related to the density of infection.

It would thus appear that the host/glochidial interaction maybe regulated by density-dependent factors controlling both the infection phase and parasite induced host mortality. The importance of these factors have been discussed by Anderson and May (1978).

In common with the other ectoparasites dealt with in this study, glochidia are not restricted to one particular host species. A list of host records for glochidia in the British Isles is given in Table 20 Because some records do not indicate which species of glochidia occur, they are grouped together as Anodonta sp. Glochidia are found on twenty species of freshwater fish.

Unlike the leeches and A.foliaceus, in which attack is largely dependent upon the searching behaviour of the parasite, glochidia rely on different mechanisms. The present section investigates the use of a different strategy of attachment and parasitism by glochidia larvae.

Materials and methods

See Section II. 1,2,3 (e) i,ii,iii (f). (pages 7 - 21).

Results

The following demonstrate experimental infections when glochidia are exposed to G.aculeatus.

Table 20

Host records for glochidia larvae of Anodonta species

Fish species	Reference
Salmo trutta (L.)	Wootten (1973) Campbell (1974)
Salmo gairdneri (Rich.)	Wootten (1973)
Thymallus thymallus (L.)	Davies (1967)
Esox lucius (L.)	Campbell (1974)
Cyprinus carpio (L.)	Lee (1977)
Carassius carassius (L.)	Lee (1977)
Gobio gobio (L.)	Lee (1977)
Tinca tinca (L.)	Dartnall and Walkey (1979)
Abramis brama (L.)	Mishra and Chubb (1969)
Phoxinus phoxinus (L.)	Present study
Rutilus rutilus (L.)	Mishra and Chubb (1969) Wootten (1973) Lee (1977)
Leuciscus cephalus (L.)	Davies (1967)
Leuciscus leuciscus (L.)	Davies (1967)
Noemacheilus barbatulus (L.)	Present study
Anguilla anguilla (L.)	Lee (1977)
Gasterosteus aculeatus (L.)	Dartnall (1972) Dartnall, Lewis and Walkey (1972) Campbell (1974) Dartnall and Walkey (1979) Present study
Pungitius pungitius (L.)	Dartnall (1973) Wootten (1973)
Perca fluviatilis (L.)	Mishra and Chubb (1969) Wootten (1973) Campbell (1974) Lee (1977)
Gymnocephalus cernua (L.)	Wootten (1973) Lee (1977)
Cottus gobio (L.)	Present study

(a) Dispersion

The dispersion of glochidia on G.aculeatus is shown in Figure 43 and analysed in Table 21 . The negative binomial distribution proved to be the best model and provided the best fit to the data (represented as circles in Figure 43). The goodness-of-fit of this distribution was confirmed by χ^2 and the statistic U. This is the difference between the sample estimate of variance and the excepted variance in the negative binomial. Parameters of the fitted negative binomial are given in Table 21 . The resulting distribution yielded by exposing 50 fish to 500 glochidia is thus seen to be overdispersed.

(b) Site selection

Of the 3,000 glochidia exposed to the 30 fish, 538 or 17.93 % attached. Ninety-seven percent of the fish were infected with a mean number of 17.93 per fish. The site distribution of glochidia on sticklebacks is given in Table 22. The numbers (and percentage) of glochidia attached to different sites are as follows:- Fins 219 (40.71 %), spines 35 (6.51 %), body 217 (40.33 %), buccal cavity 35 (6.51 %) and gills 32 (5.91 %). There is no significant difference between the numbers attached to the fins/spines and the body (χ^2= 2.915 at P>0.05). χ^2analysis (Table 22) indicates a significant aggregation on the lower (ventral) half of the fish, particularly on the anterior body (39.59 %), anal (17.66 %) and caudal fins (16.73 %).

(c) Change in site distribution

The resulting site selection when five sticklebacks were exposed to 100 glochidia for 24 hours at both 10 ^{o} C. and 20 ^{o} C. is given in Table 23 . Preferential sites are similar at both temperatures and to

Figure 43

The experimental frequency distribution of glochidia larvae on G.aculeatus

●—● Fitted negative binomial model

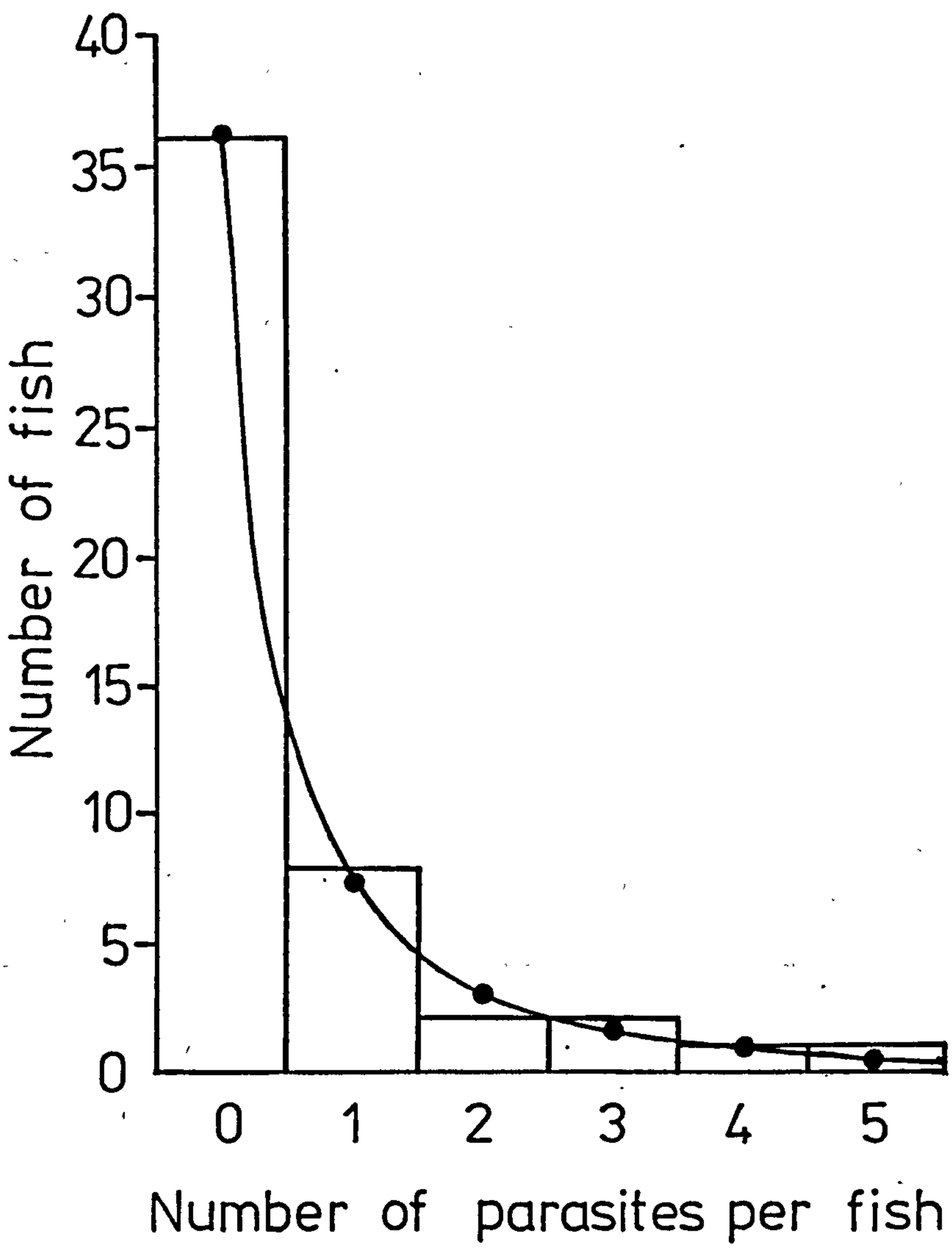

40
35
30
25
20
15
10
5
0
Number of fish
0
1
2
3
4
5
Number of parasites per fish

Table 21

Parameters and test statistics of the fit
to the negative binomial distribution

Number of fish	50
Total number of glochidia attached	27
Mean	0.54
Variance	1.22
Variance/mean ratio	2.26
k	0.355
d.f.	4
Chi-square	0.858
U	-0.14
Standard error of U	0.28

Table 22

Site distribution of glochidia on G.aculeatus

Site	Total	Position		Chi-square	Significance
		Ventral	Dorsal		
Fins	219				
Caudal	90	90	0	90.00	sign.
Anal	95	95	-	86.37	sign.
Dorsal	3	-	3		
Pectoral	31	-	-	-	-
Total (exc. pect. fins)	188	185	3	176.19	sign.
Spines	35	35	0	35.00	sign.
Body	217	214	3	205.16	sign.
Anterior	216	213	3	204.17	sign.
Middle	1	1	0	1.00	n/s
Posterior	0	-	-	0.00	-
Buccal cavity	35	-	-	-	-
Gills	32	-	-	-	-
(1)	10	-	-	-	-
(2)	12	-	-	-	-
(3)	7	-	-	-	-
(4)	3	-	-	-	-
Total (exc.gills, +buccal cavity + pectoral fins)	440	434	6	416.33	sign.

Table 23

Site selection of glochidia on G.aculeatus

Site	Total	Percentage
10°C.		
Anterior body	21	37.5
Anal fin	14	25.0
Caudal fin	12	21.4
Ventral spines	5	9.0
Pectoral fin	4	7.1
Total	56	100.0
20°C.		
Anterior body	13	40.6
Anal fin	8	25.0
Caudal fin	5	15.6
Ventral spines	3	9.4
Pectoral fin	3	9.4
Total	32	100.0

those previously discussed (V (b)). After three weeks at 20 ^{o} C. and five weeks at 10 ^{o} C.,all fish had died but at no stage were the glochidia in different positions from those initially selected, or had any left the fish.

(d) Larval infectivity

The mean number of glochidia attaching to the fish over a six day period is given in Figure 44 . The results indicate agreement with the Poisson series (Table 24). Consequently, possible variations in fish susceptibility does not account for the change in infectivity over a period of six days and is clearly some function of the ageing process of glochidia. Infectivity declines rapidly with age.

Discussion

On release from the adult, glochidia are dependent upon water currents for transfer and thus the probability of encountering a host is fairly low and largely dependent upon chance events. Part of the strategy enhancing infection therefore lies in the fact that glochidia are able to infect and develop on a large number of fish species. The parasitic stage also provides a means of dispersal from an adult having limited powers of locomotion. While glochidia can survive in the free living phase for periods of up to one week, it is during this phase that heavy larval mortality will occur. Glochidia are reported to be preyed upon by Chaetogaster diaphanus (Gruithuisen) and Stenostoma giganteum (Highley) (Howard and Anson, 1922).It is likely that these predators only play a minor role in controlling the numbers of free living glochidia.

Analysis of the mean number of glochidia attaching to

Figure 44

The mean number of glochidia of a given age which attach to the fish over a six day period.

● = Observed means. Vertical bars represent 95% confidence limits.

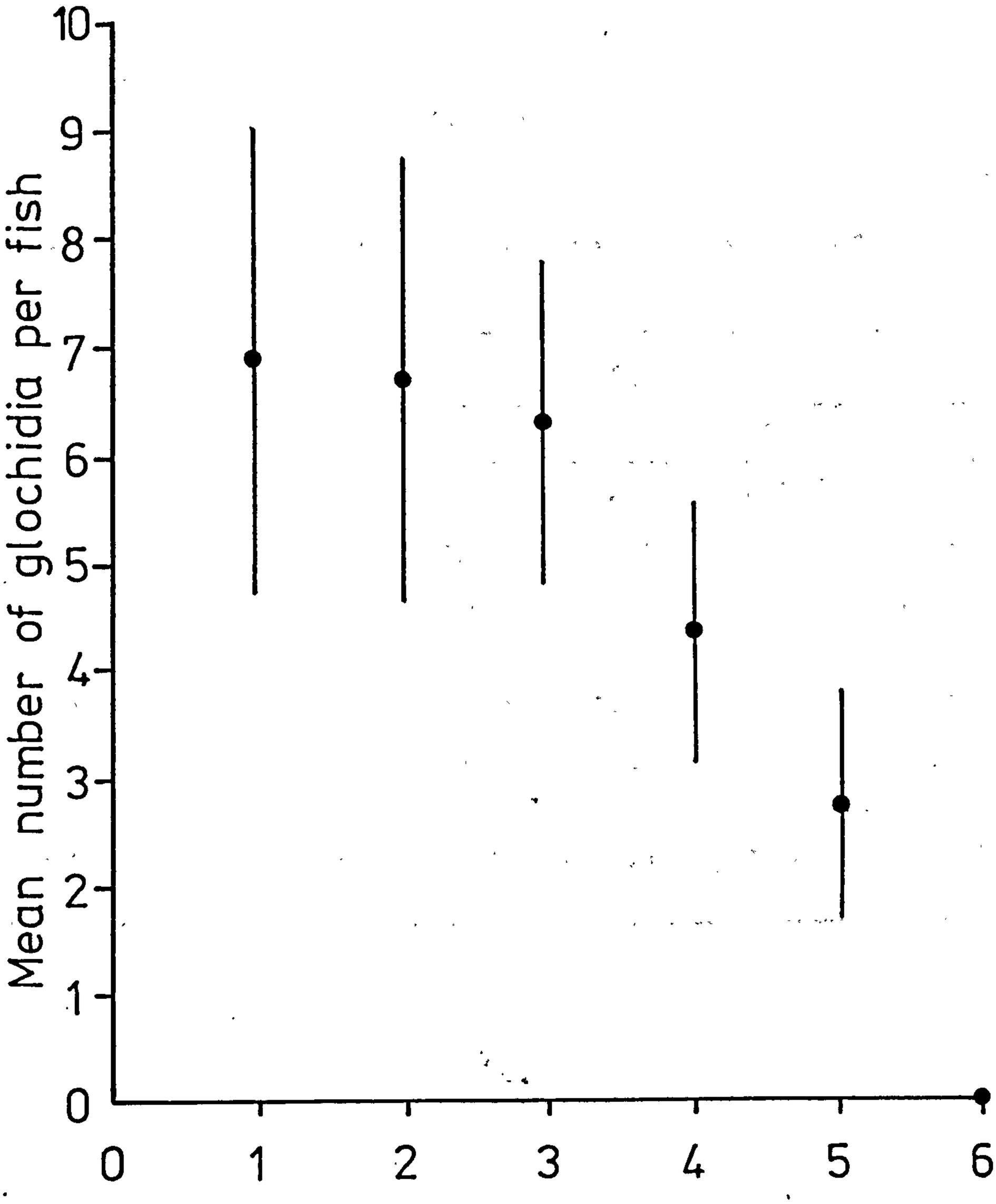

Mean number of glochidia per fish
0
1
2
3
4
5
6
7
8
9
10
Time (days)

Table 24

Infection of glochidia on G.aculeatus as a function of age

	Days				
	1	2	3	4	5
Mean	6.9	6.7	6.3	4.4	2.8
Variance	11.88	10.46	5.57	3.38	2.84
Variance/mean ratio	1.72	1.56	0.88	1.16	1.01
Chi-square	15.50	14.05	7.96	6.91	9.13
Significance (at P 0.05)	n/s	n/s	n/s	n/s	n/s
95% confidence limits	± 2.17	± 2.04	± 1.49	± 1.16	± 1.06

G.aculeatus over a six day period indicates that there is a distinct decrease in infectivity in relation to age. Even after twenty-four hours exposure, the mean number of glochidia attaching is relatively low, due to the passive mode of infection. The decline in infectivity may be directly related to the breakdown of endogenous energy reserves. The long survival period is associated with a relatively limited activity and consequent conservation of these energy reserves. Little is known about the daily production of larvae by the adult and how their continual release may counterbalance the decreased infectivity of those previously released.

The experimental dispersion pattern of glochidia on G.aculeatus was adequately described by the negative binomial distribution and found to be overdispersed. The dispersion of many parasites may be described by the negative binomial distribution (Crofton, 1971b; Pennycuick, 1971; Boxshall, 1974a; Kennedy, 1975a) which can be generated by a number of biological and physical processes (Anderson and May, 1978). It is of importance therefore, to consider whether the dispersion pattern produced by glochidia is a consequence of glochidial attack or simply caused by heterogeneity amongst the host population in their susceptibility to infection.

Overdispersed distributions of larval parasites are often explained by the fact that behavioural responses of infective larvae bring aggregations into close proximity with their potential hosts (Boxshall, 1974a). Aggregation in this sense implies non-random search created by a specific behavioural strategy. It has been mentioned that glochidia are passive in the transmission phase and so the attack strategy is presumably fortuitous. The release of larval aggregations(joined by byssus threads) from the adult bivalve will result in a clumped

distribution of glochidia in the host's habitat. This factor, and differences in fish behaviour is likely to produce a situation where certain fish moving into aggregations of larvae are more likely to be infected than others. The difference in infection levels may thus depend on the size of the fish as well as the particular host species with its characteristic shoaling behaviour. Overdispersed distributions have been described for natural infections of glochidia on sticklebacks (Dartnall and Walkey, 1979).

The experimental distribution could be generated by several factors unrelated to glochidial attack. The experimental design in terms of the ratio of water/fish/parasites used could theoretically produce underdispersed,random or overdispersed distributions. Glochidia were not attached by byssus threads (thus not aggregated) in the experiments and it thus seems more likely that the distribution produced was caused by differences in the susceptibility or behaviour of fish. Under the present experimental regimes, sticklebacks were not previously exposed to parasites. Thus the possibility of an immune response occurring can be discounted.

Unlike the leeches and A.foliaceus, death of hosts parasitised by glochidia will result in parasite mortality. Mortality amongst fish caused by heavy infections will also exert some influence on the regulation of host/parasite populations.

Preferential sites of glochidia on sticklebacks are the fins and head region, with little infection on the gills/buccal cavity. Similar results have been demonstrated for natural infections of glochidia on sticklebacks (Dartnall and Walkey, 1979). The spatial distribution on the gills closely resembles that described for brown trout (Paling, 1968), which was related to a greater water flow through the second

and third pairs of gills during respiration. The aggregation of larvae on the lower half of the body (particularly in the head region) and fins could be related to the behavioural activity of the fish. The site distribution is likely to be a consequence of stickleback feeding activity in relation to the swimming behaviour of the fish. In this case, the lower regions of the fish, particularly those which are likely to come into contact with glochidia first, such as the anterior body, ventral spines, anal and caudal fins will become infected. The site selection in the mouth and buccal cavity maybe a consequence of predation and the presence of larvae on the gills could then be attributed to respiratory movements once larvae have entered the mouth.

Glochidia do not move from their initial attachment sites once cyst formation is complete. This again is reflected by the passive mode of attachment. Metamorphosis of glochidia was not observed in the present study.

VI. FINAL DISCUSSION

Attempts by many authors to classify the spectrum of inter-specific animal and plant relationships have yielded a plethora of terms with considerable variation in definitions. Depending upon the origin and emphasis of the respective definitions, a particular association may be categorised in several ways. Leeches, for example, may be considered as predators, vectors, obligate, periodic or temporary parasites.

The present work is concerned with some basic factors controlling the feeding (or attack) strategies of some non-specific fish ectoparasites. The host/parasite relationships selected as models for the study exhibit varying degrees of complexity, particularly when temporal changes are considered.

Rothschild and Clay (1952) stated "Although so-called obligate parasites are at some period of their lives dependent upon a host, many of them are able to spend long spells in the free state and it is normal for them to do so. These animals are generally referred to as periodical parasites, whereas those which are dependent on a host during one stage of the life cycle are designated as temporary parasites".

It is convenient to commence a consideration of the relationships between fish and their ectoparasites on this basis. In this event, leeches and Argulus may be defined as periodical parasites and glochidia as temporary parasites.

These designations may be amplified when the mechanisms controlling infection are considered. In the leeches and Argulus, the parasitic phase is controlled by two basic population processes, namely immigration and emigration. The situation is slightly different for glochidia if infection levels are influenced by death of parasites whilst

on the host (Arey, 1932b).

Immigration and death have been implicated by Anderson (1974) as the major processes controlling infection levels in definitive hosts for parasites with "direct" life cycles (Figure 45). Such parasites presumably exhibit a permanent or sub-permanent parasitic phase (Figure 45) and amongst fish ectoparasites are exemplified by the Monogenea or Copepoda.

The factors influencing infection by periodical parasites are summarised in Figure 46. Controlling factors in glochidial life cycles are also included.

Preliminary comparison of the two systems indicate the following features:-

1) In permanent and temporary parasites, death of the host, whether due to natural causes or to parasite-induced host mortality, causes death of parasites. By comparison, death of the host does not induce mortality amongst periodical parasites since such parasites are able to leave the dying host and re-infect a new host.

2) Parasites with "direct" life cycles experience adult mortality in or on the definitive host whereas periodical parasites usually die in the free-living phase. In the case of Anodonta, larval death may be caused by a host response to infection (Arey, 1932b). Such responses have not yet been established for leeches and Argulus although they may be responsible for emigration immediately prior to death of parasites.

3) In aquatic habitats, egg production by ectoparasites occurs on the host in direct life cycles, with eggs subsequently being released into the water. In all species currently being considered, egg-laying occurs in the free living stage.

In many respects, the life cycle of A.cygnea is similar to that of parasites possessing direct life cycles. It also, however, shares

certain characteristics with the periodical parasites. It is therefore valid to examine further the distinction between periodical and temporary parasites. In the fish leeches and Argulus, all stages, except eggs or cocoons, can parasitise a number of hosts, exhibiting regular phases of immigration and emigration, hence free living and parasitic existences. This may be construed as a major characteristic of periodical parasitism.

In the case of leeches, the major stimulus for parasitism is that of hunger. In Argulus, it is less easy to establish that hunger plays a major role, the parasitic phase being irregular and unpredictable. Further analysis of the factors controlling Argulus infections is obviously needed. In terms of basic attack strategy, the four ectoparasites studied show varying levels of activity. At one end of the spectrum, glochidia are purely passive and are dependent upon water currents for their dispersal. Infection, therefore, is largely influenced by the behavioural activity of the host species. H.marginata, exhibits a slow searching pattern only when hungry and attack is usually restricted to bottom dwelling fish. P.geometra, when hungry, is much more active than H.marginata during searching, especially in relation to its speed of reaction to fish. Attack is therefore principally upon mid-water fish species. At the other end of the spectrum, Argulus spends a majority of its time actively swimming, although this is not necessarily part of a searching activity for new hosts.

Whilst density-dependent factors do not apparantly influence the searching activity of Argulus or Piscicola, high densities may well exert a measure of control upon the populations in the parasitic phase. Once the carrying capacity of the parasite population is reached, intraspecific competition for space and/or nutrients must occur. Such competition will be reflected in reproductive rates of the parasites leading perhaps to reduced fecundity and a general decline in the numbers of infective stages. Such effects do not appear to characterise the infection patterns exhibited

by glochidia.

Thus, incidence of infection, whilst perhaps being an accepted parameter for examination of parasites with direct life cycles, is a completely unreliable statistic for examining the biology of temporary or periodic parasites.

Figure 45

Diagrammatic flow chart of parasites with direct life cycles (from Anderson, 1974)

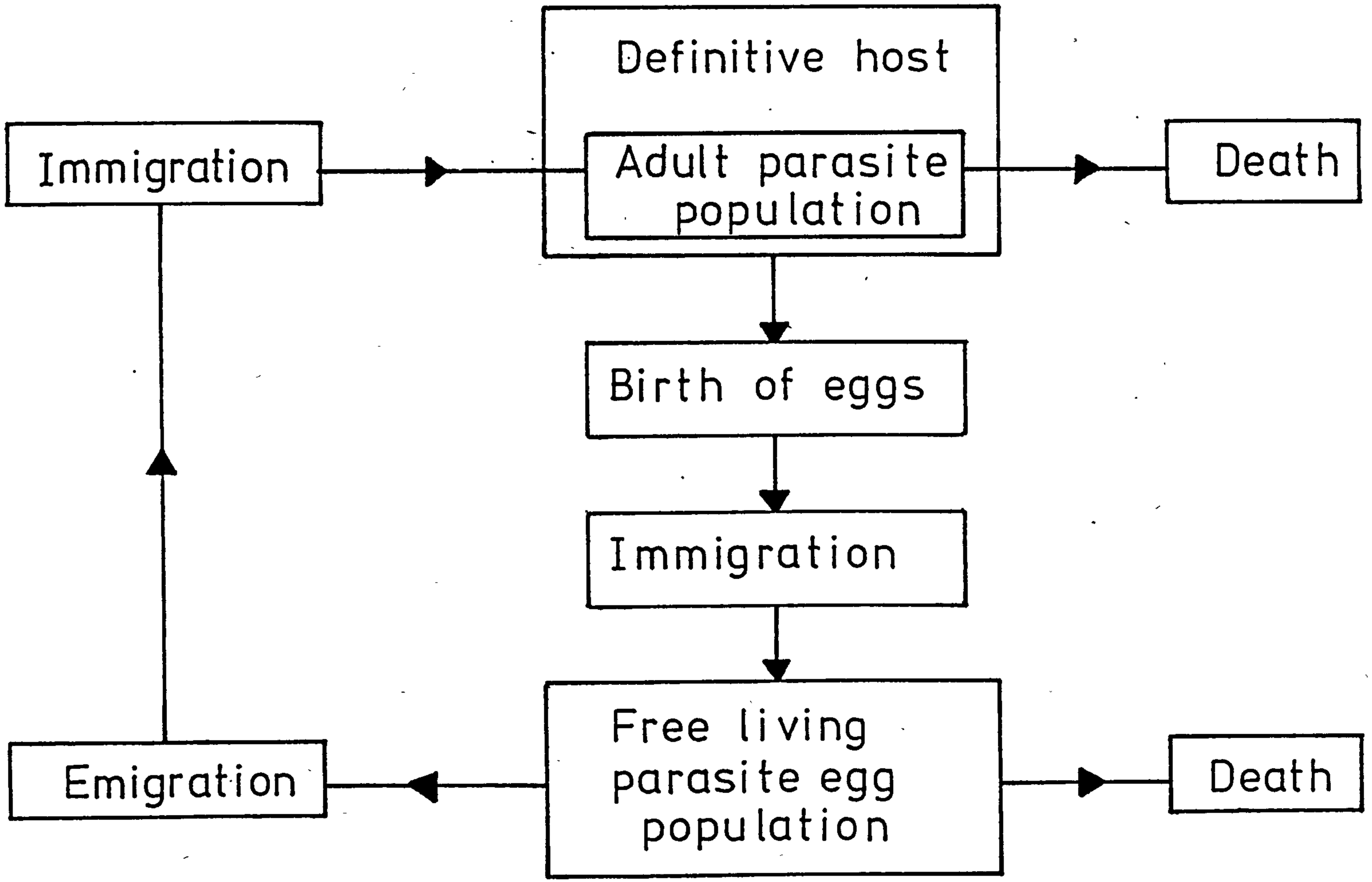
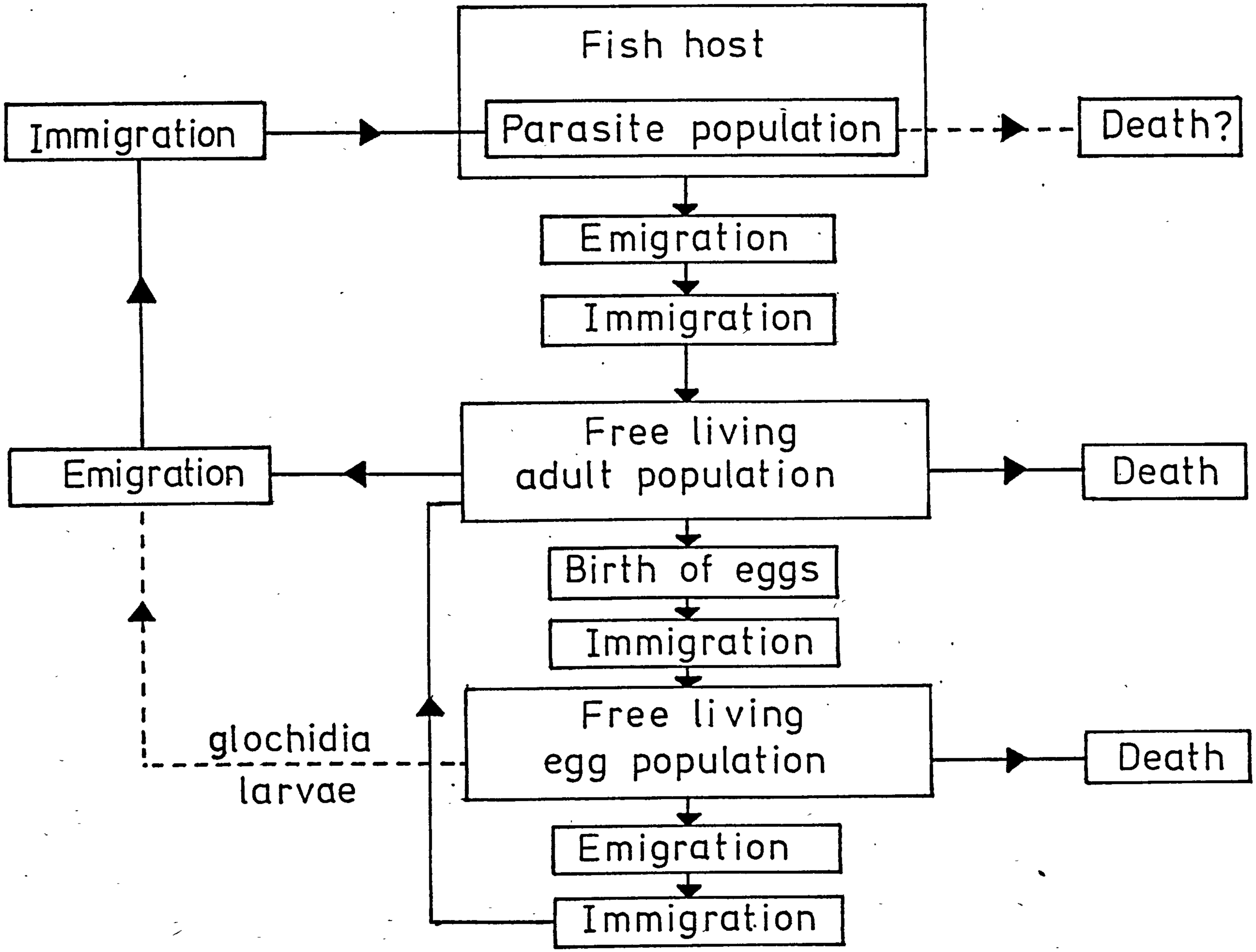

Definitive host
Immigration
Adult parasite population
Death
Birth of eggs
Immigration
Free living parasite egg population
Emigration
Death

Figure 46

Diagrammatic flow chart of parasites with

periodical life cycles


Fish host
Immigration
Parasite population
Death?
Emigration
Immigration
Free living
adult population
Emigration
Death
Birth of eggs
Immigration
Free living
egg population
glochidia
larvae
Death
Emigration
Immigration

PLATES

Plate 1

Site selection of *Argulus foliaceus* on *C.carassius* at 20°C.(After 3 - 4 weeks).

Head region.

Plate 2

As above-closer view of head region.

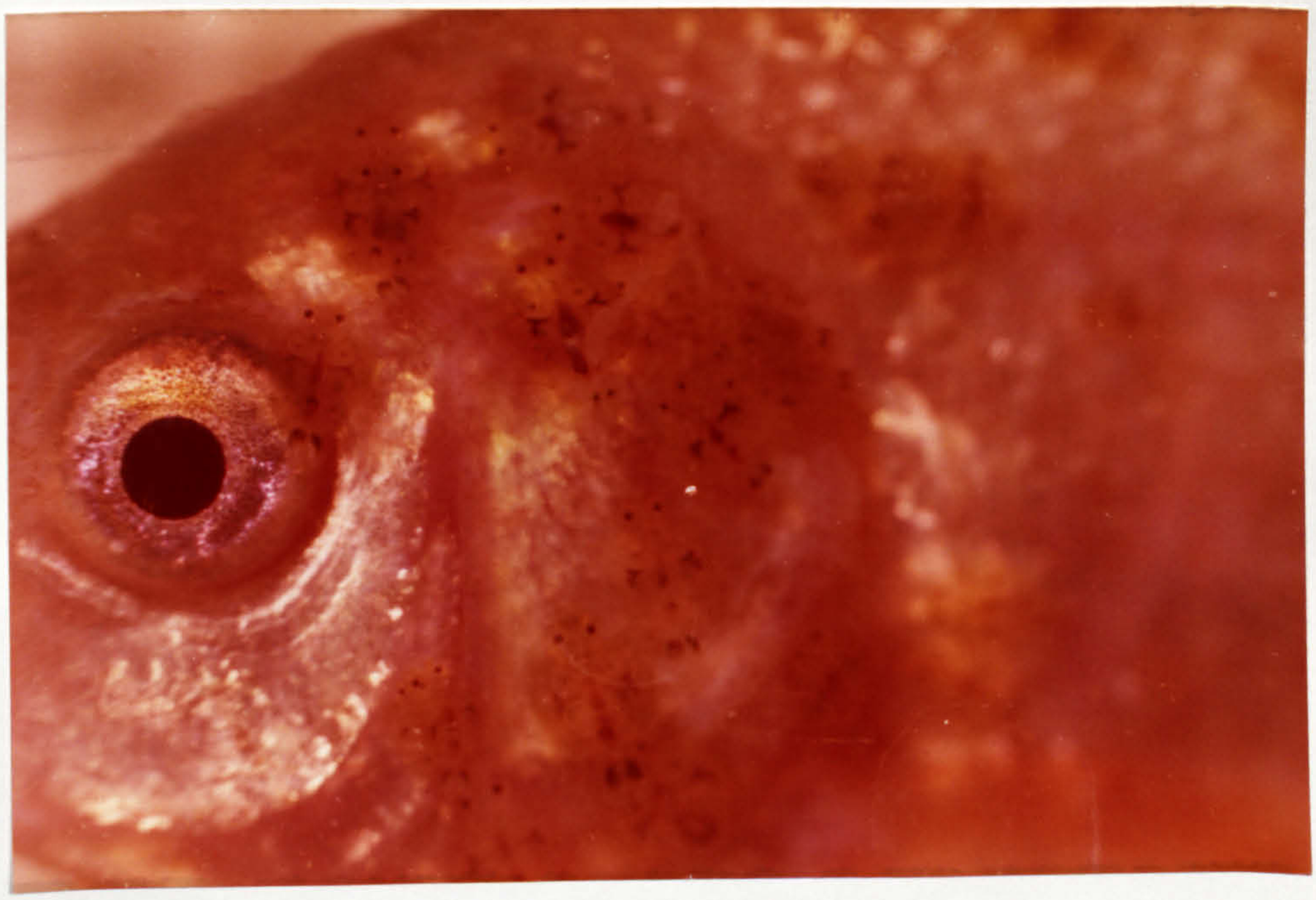


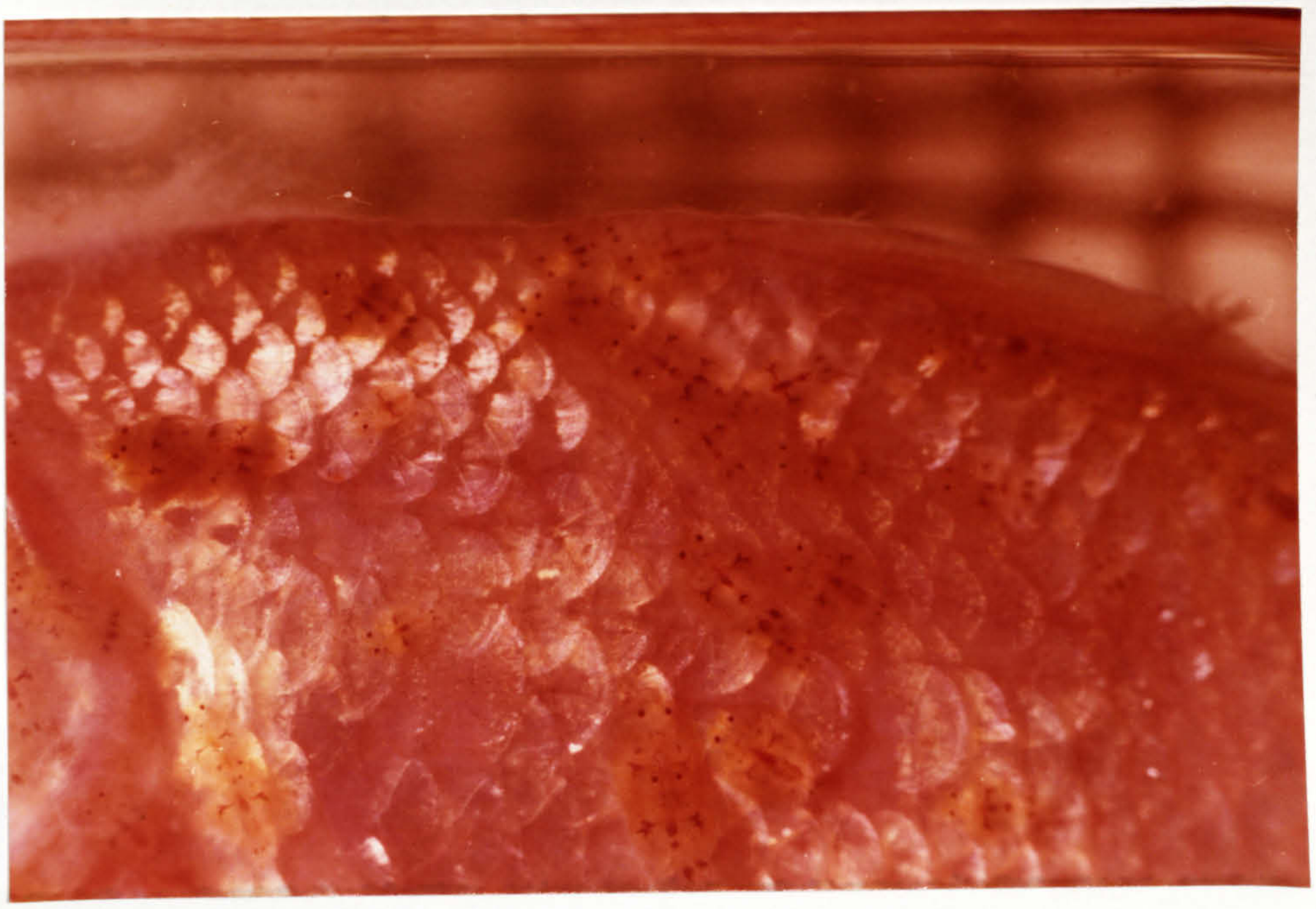
Plate 3

Site selection of *Argulus foliaceus* on *C.carassius* at 20° C. (After 3 - 4 weeks).

Middle region.

Plate 4

As above - closer view of middle region.



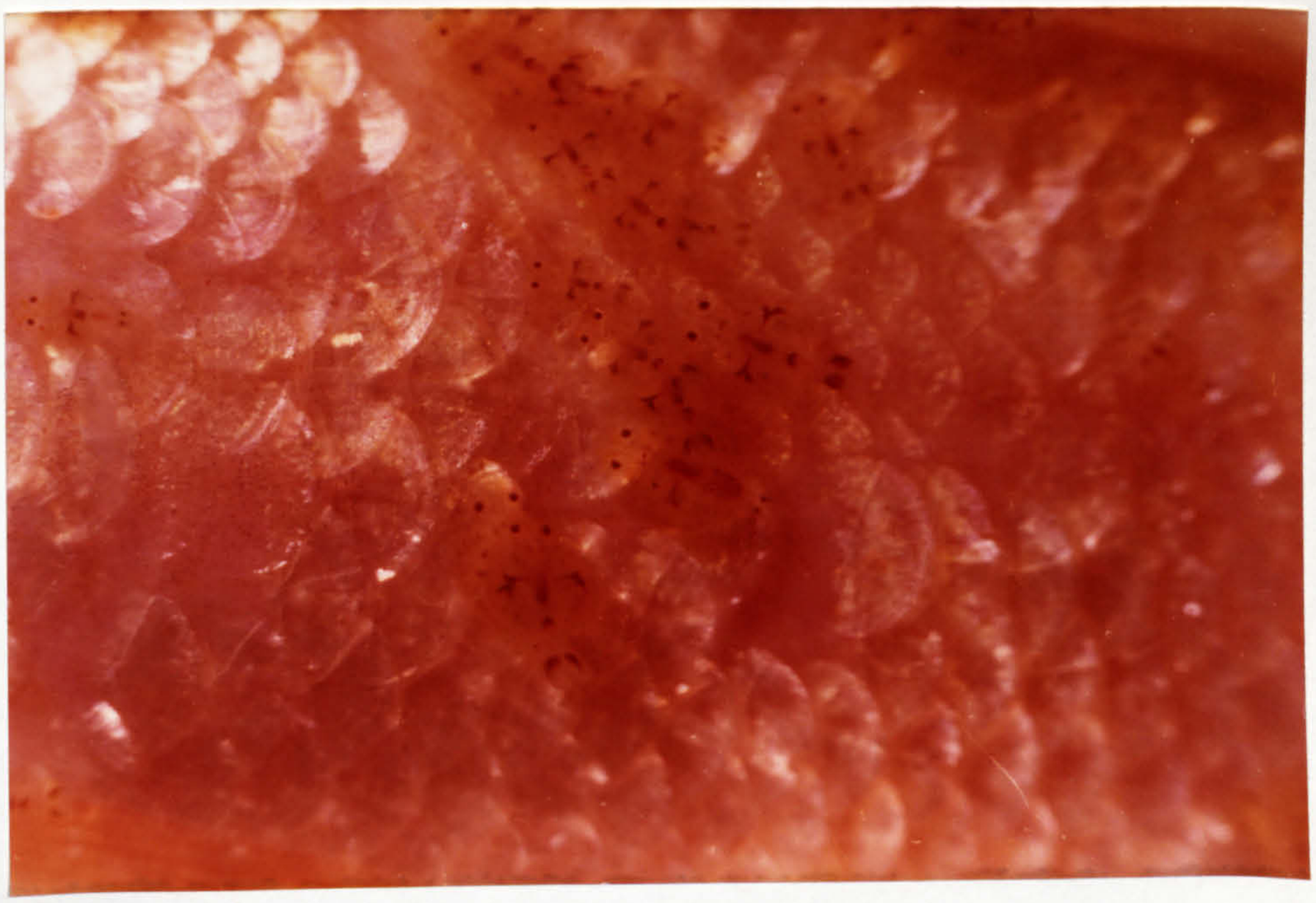
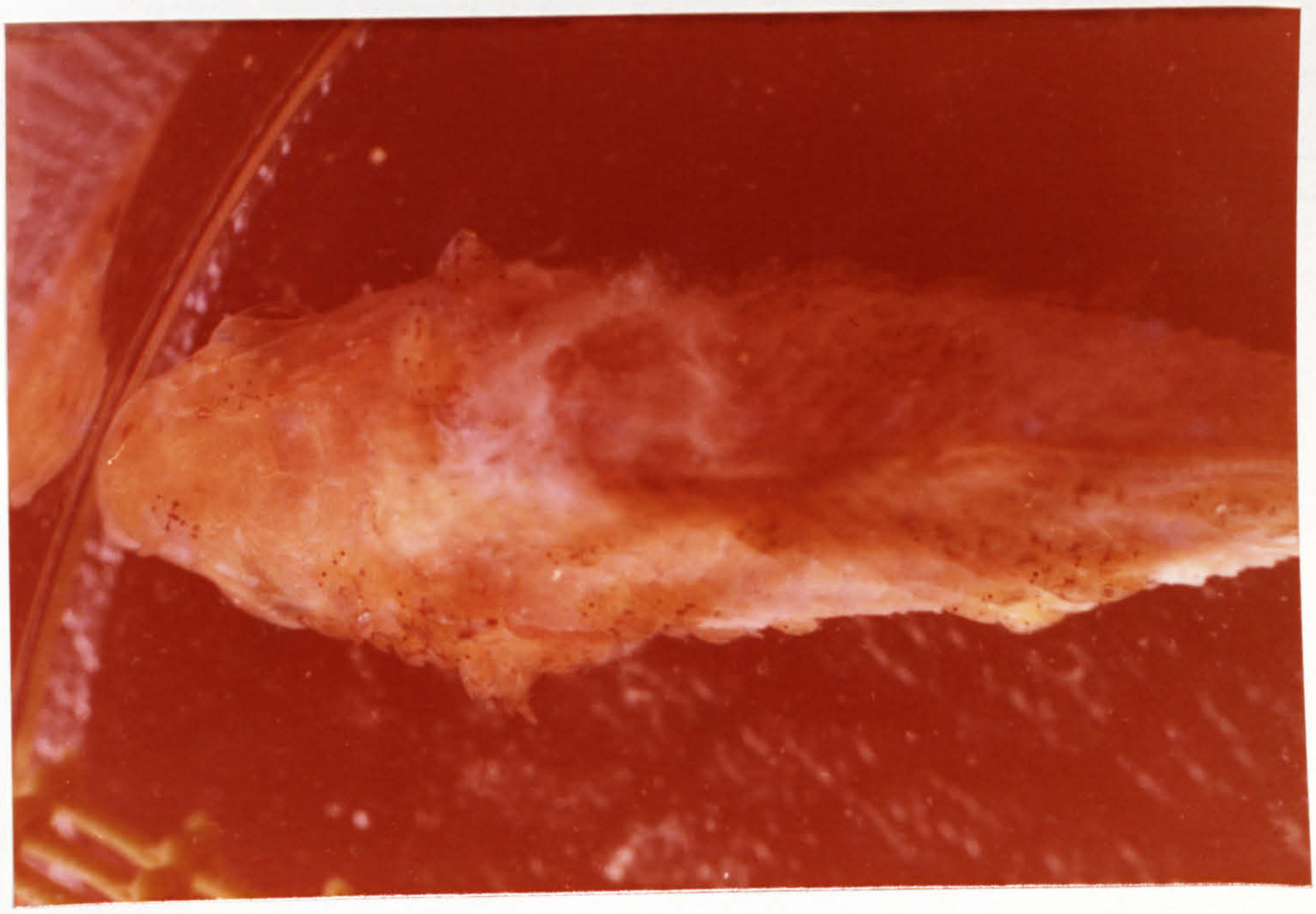


Plate 5

Dorsal view of head and middle region of *C.carassius* showing the distribution of *A.foliaceus* in areas devoid of fungal infection (indicated by white patches).

Plate 6

Dorsal view of middle and posterior regions of *C.carassius* showing the distribution of *A.foliaceus* in areas devoid of fungal infection (indicated by white patches).



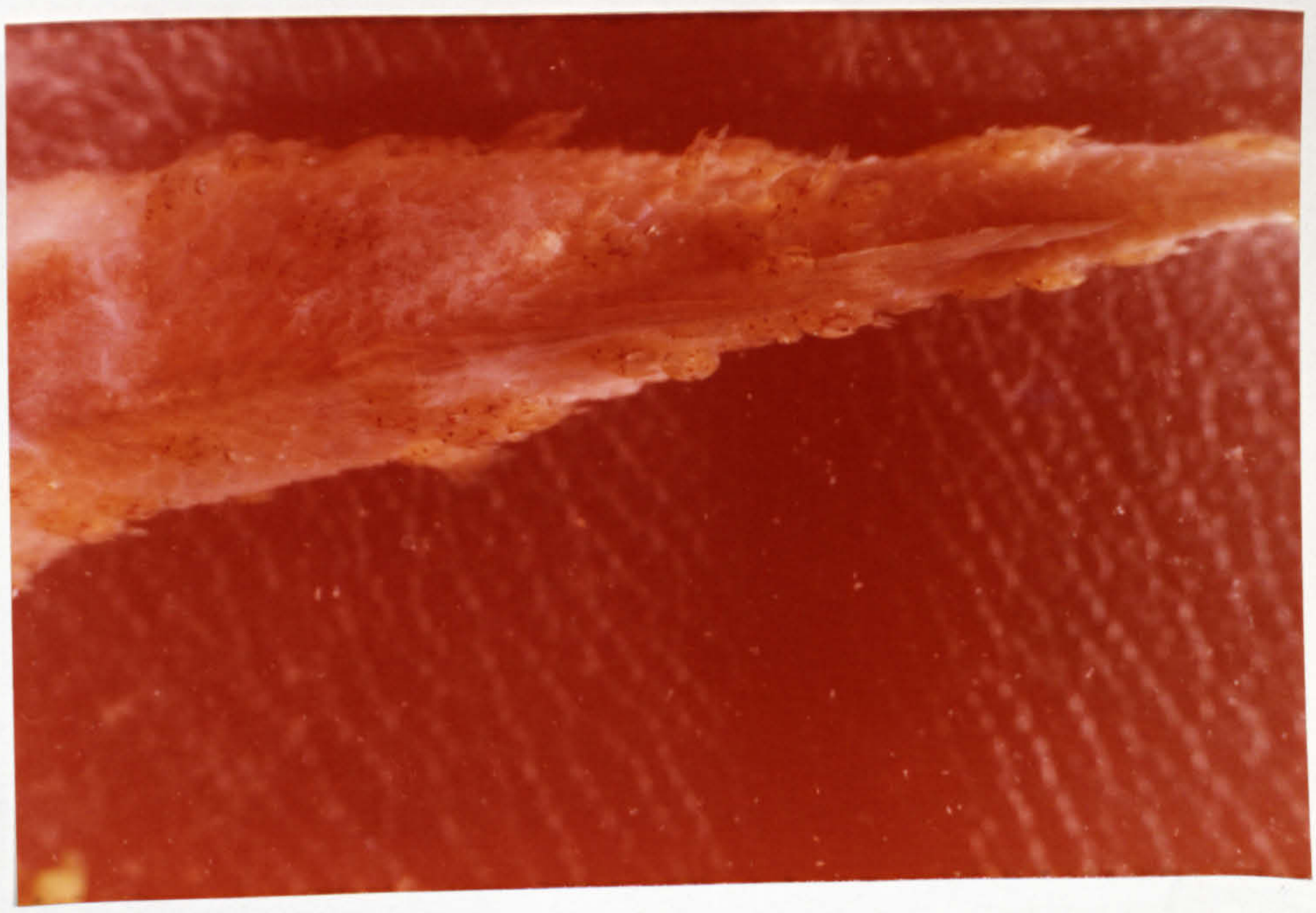
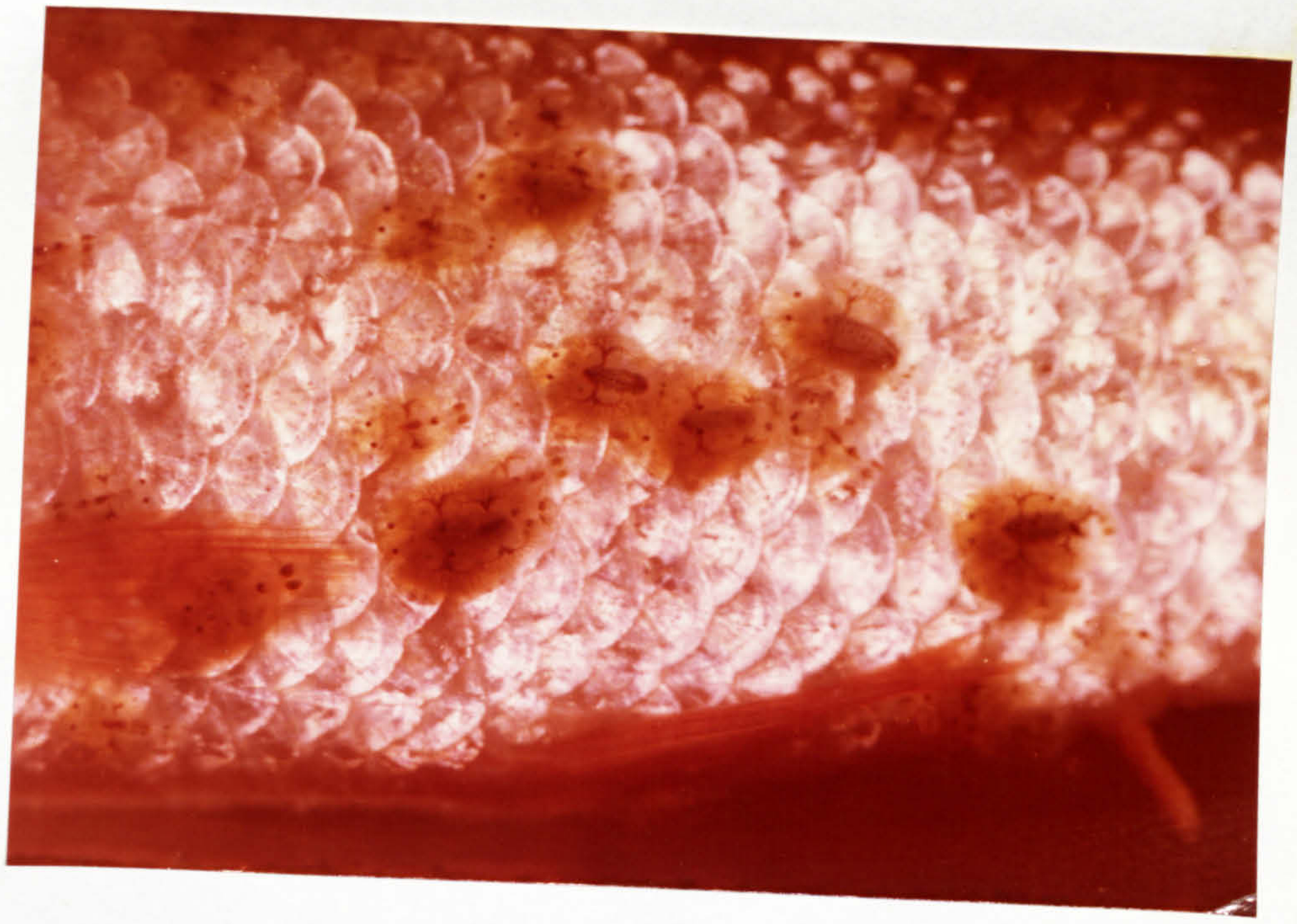


Plate 7

Mating and feeding activity in A.foliaceus

Plate 8

As above - closer view.



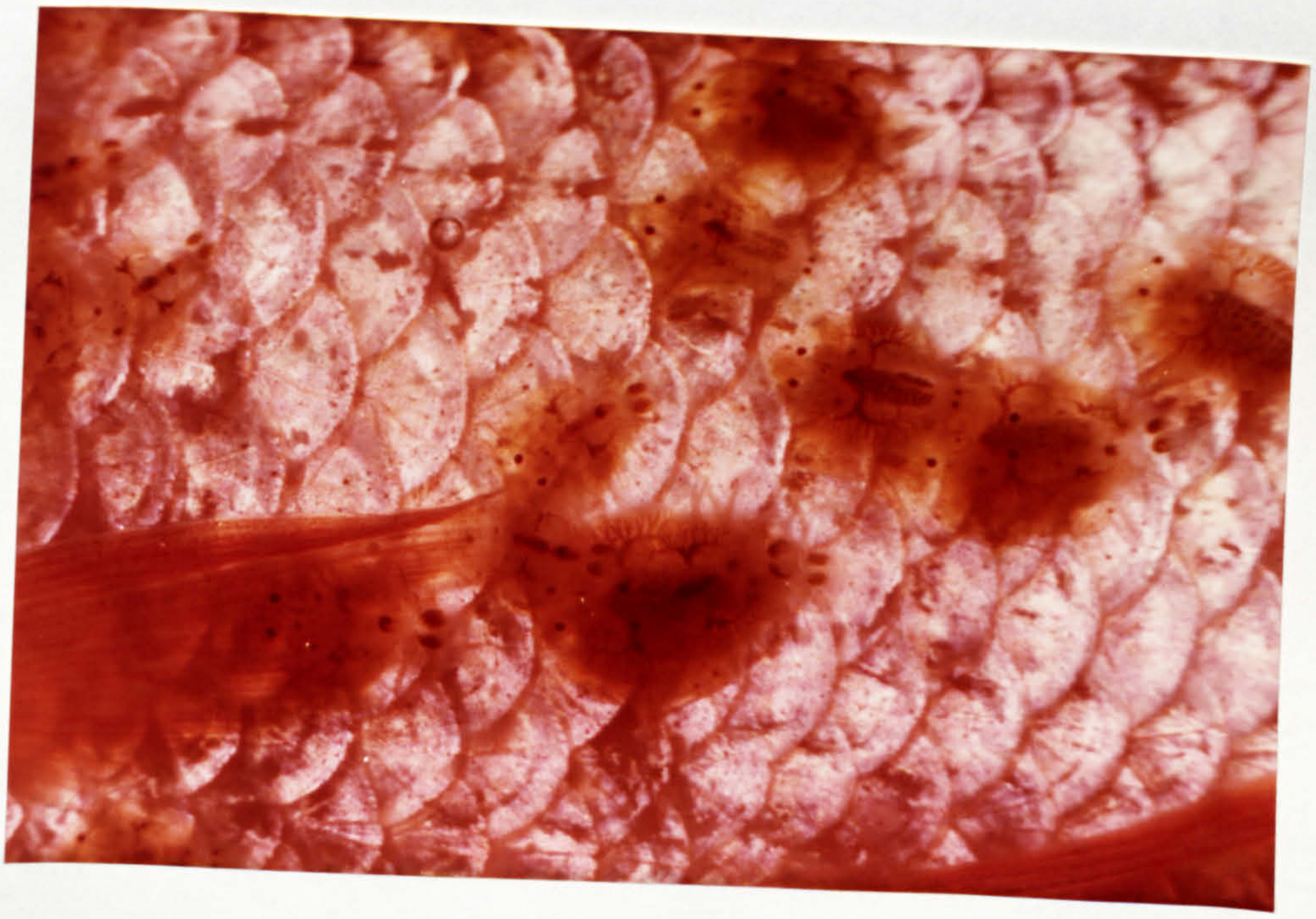


Plate 9

Close up of female A.foliaceus to show gut caecae filled with blood and egg sacs.

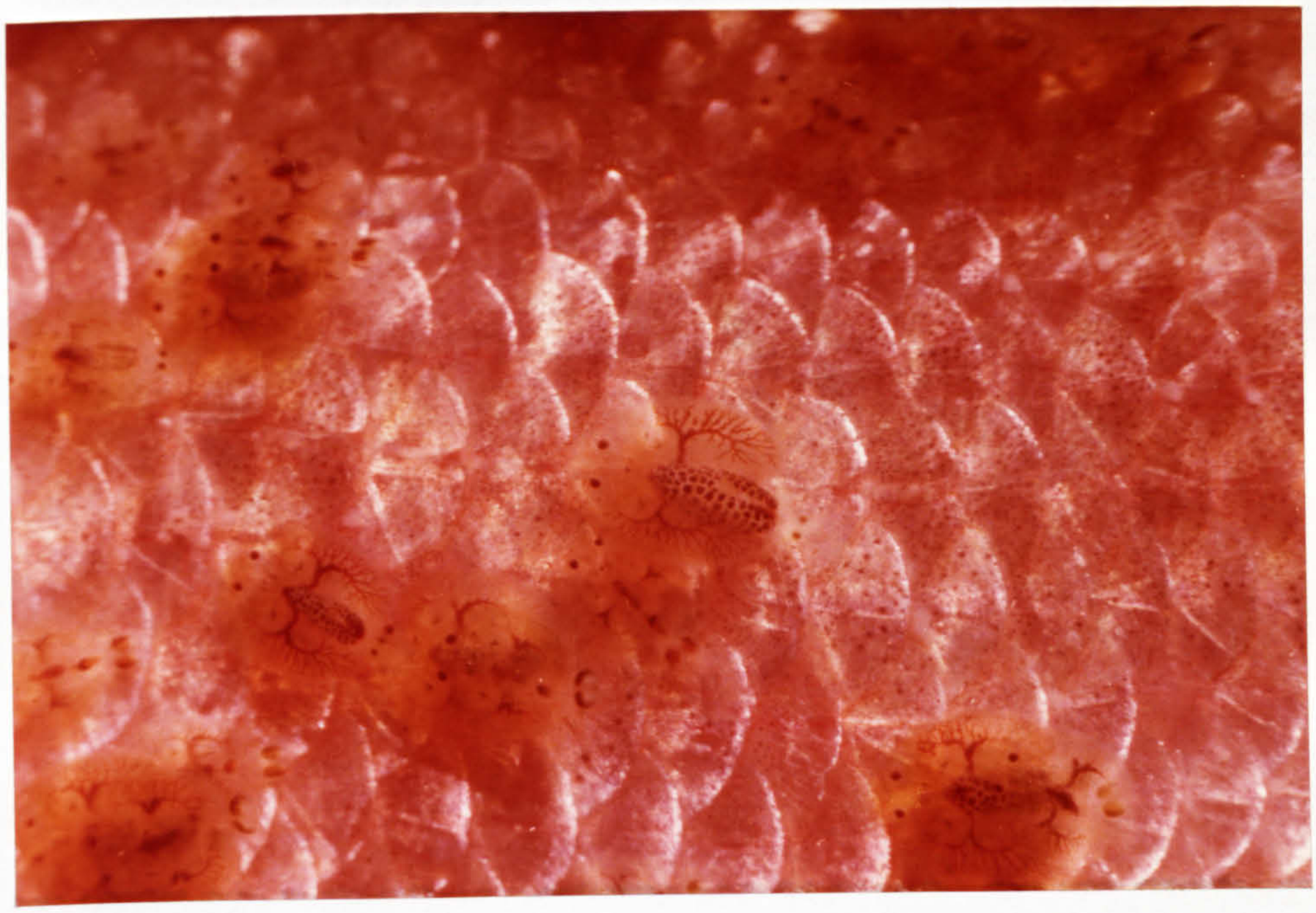
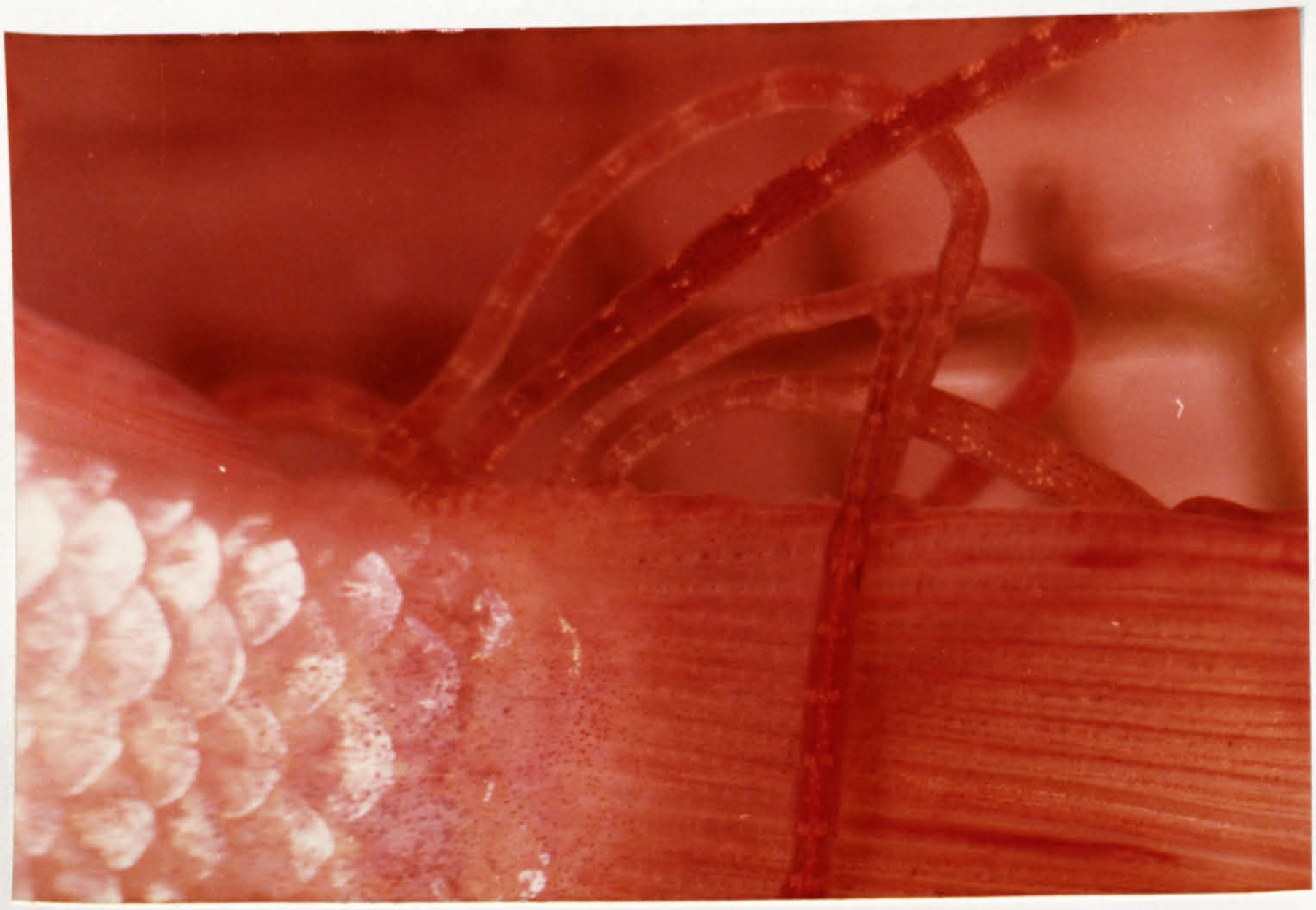


Plate 10

P.geometra feeding on the caudal fin to show differences in the amount of blood in the gut of individual leeches.

Plate 11

As above - closer view.



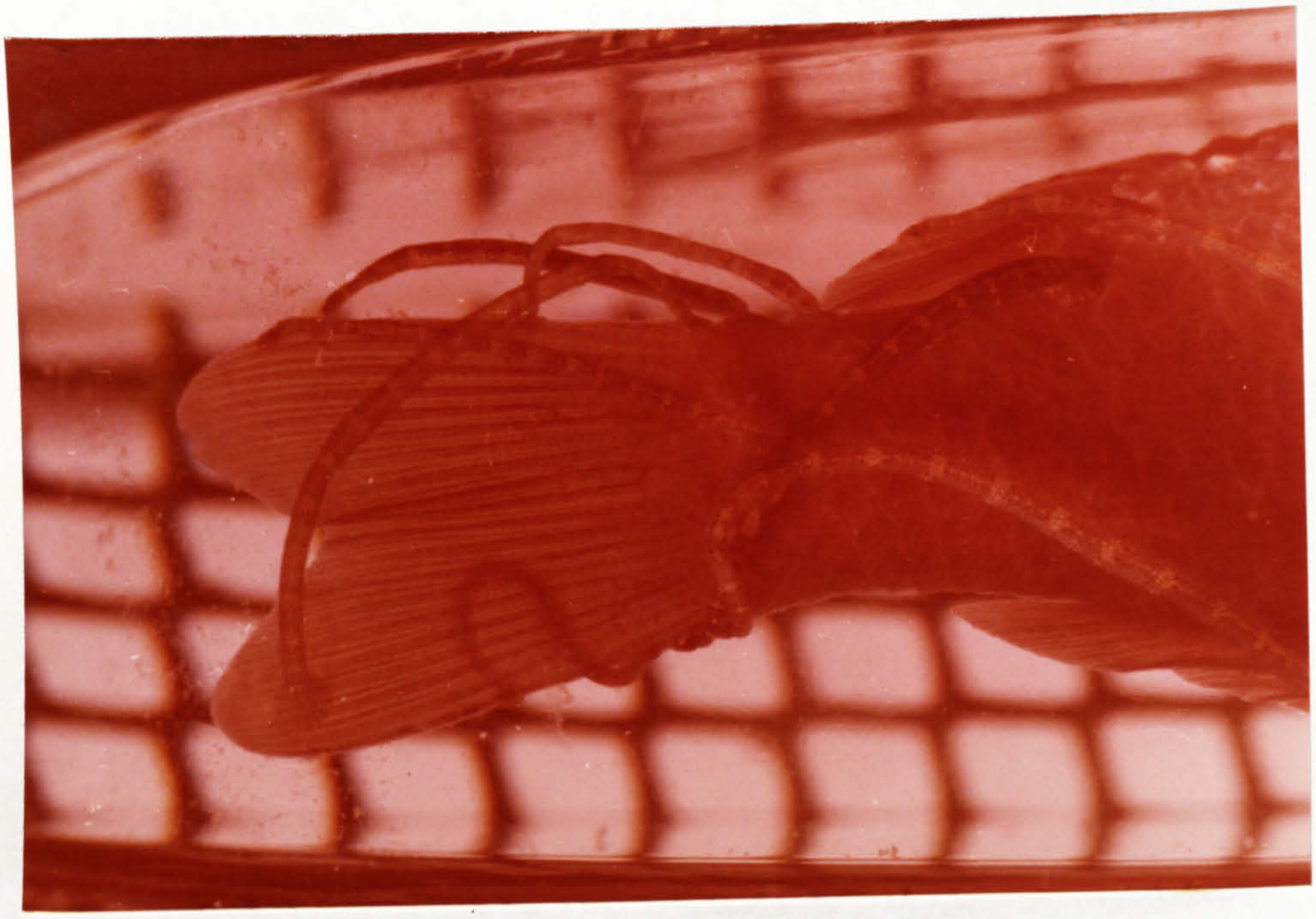


Plate 12

Copulation of P.geometra on C.carassius.

Plate 13

Whole view of C.carassius to show the initial attachment of P.geometra.

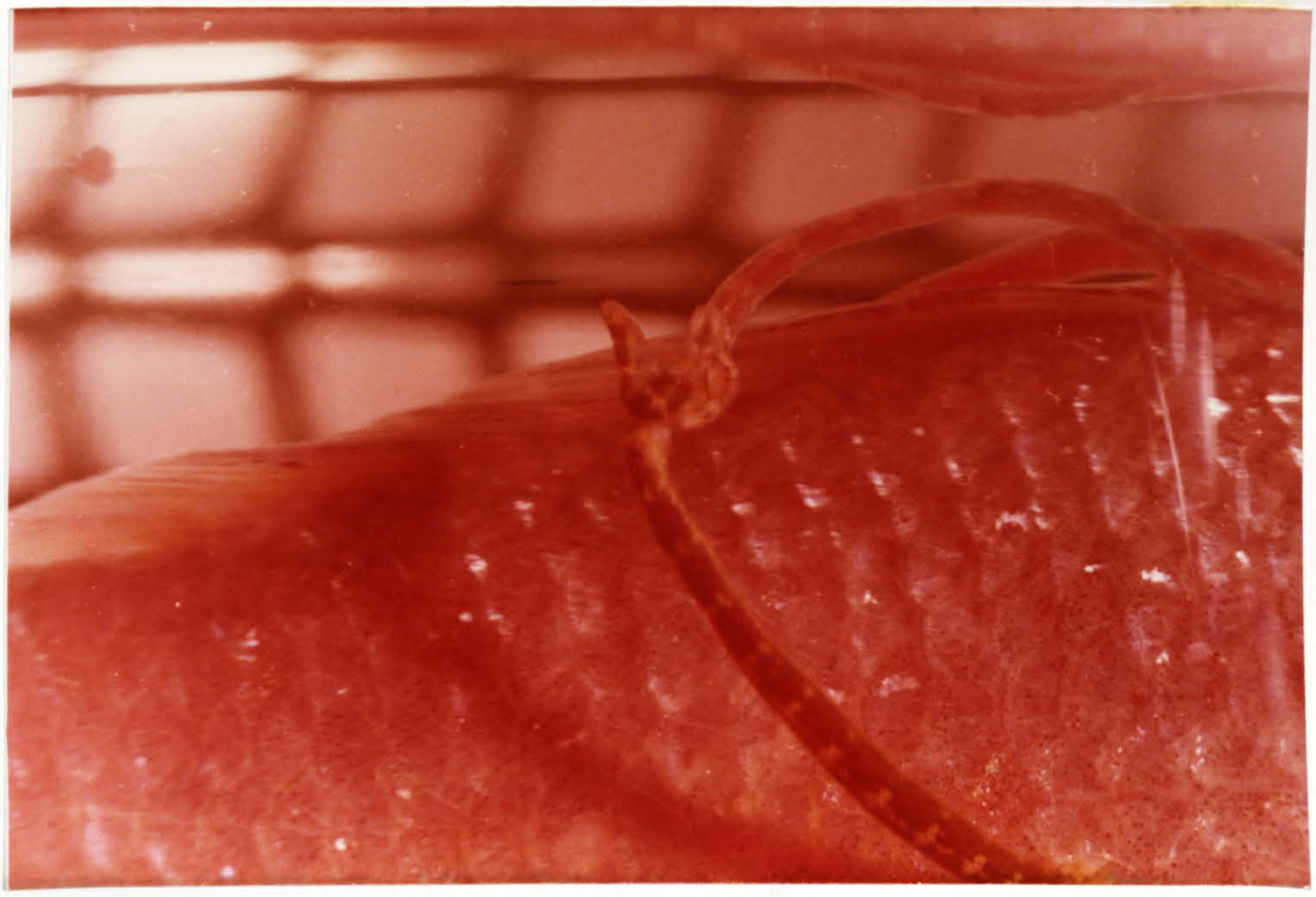



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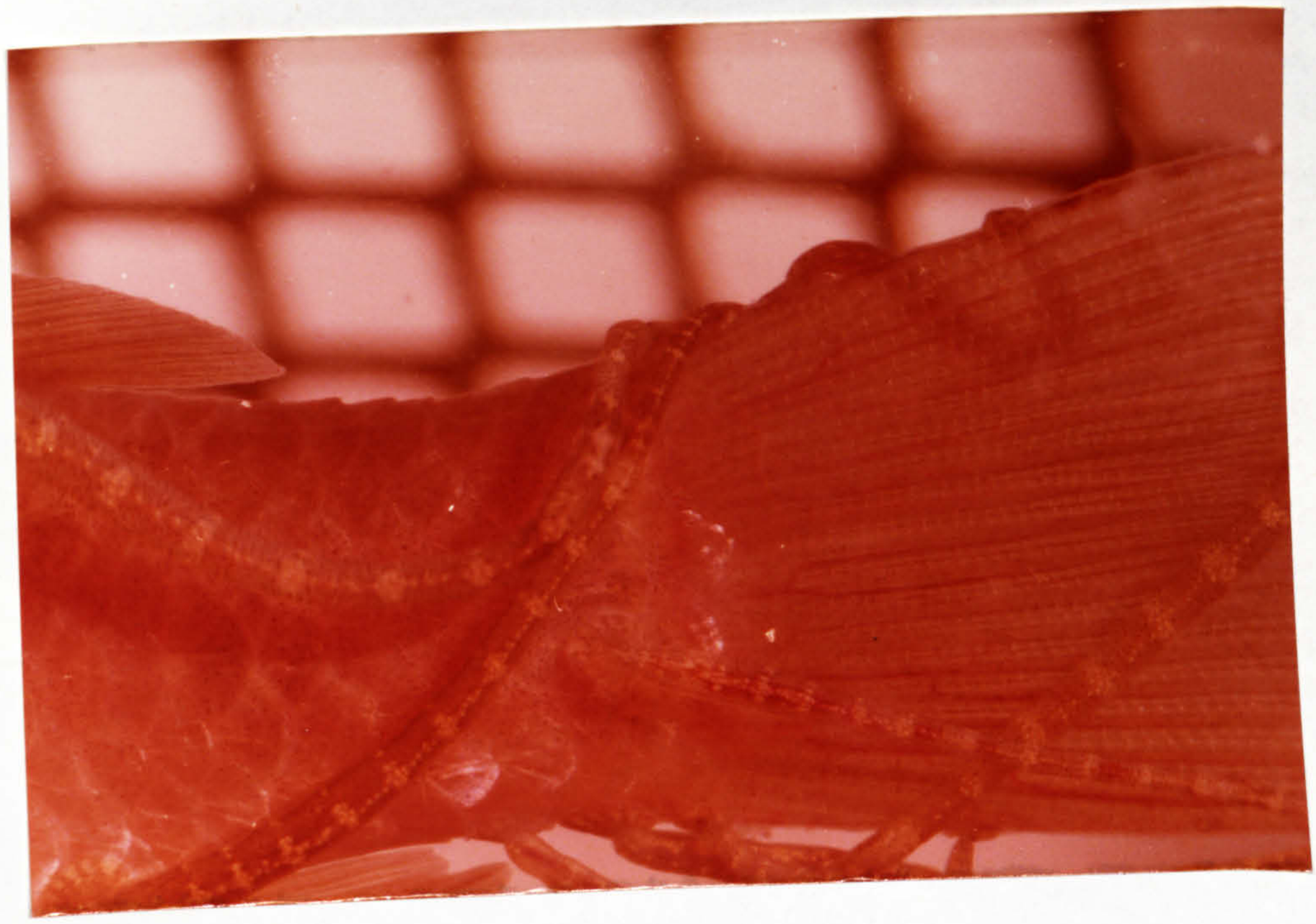
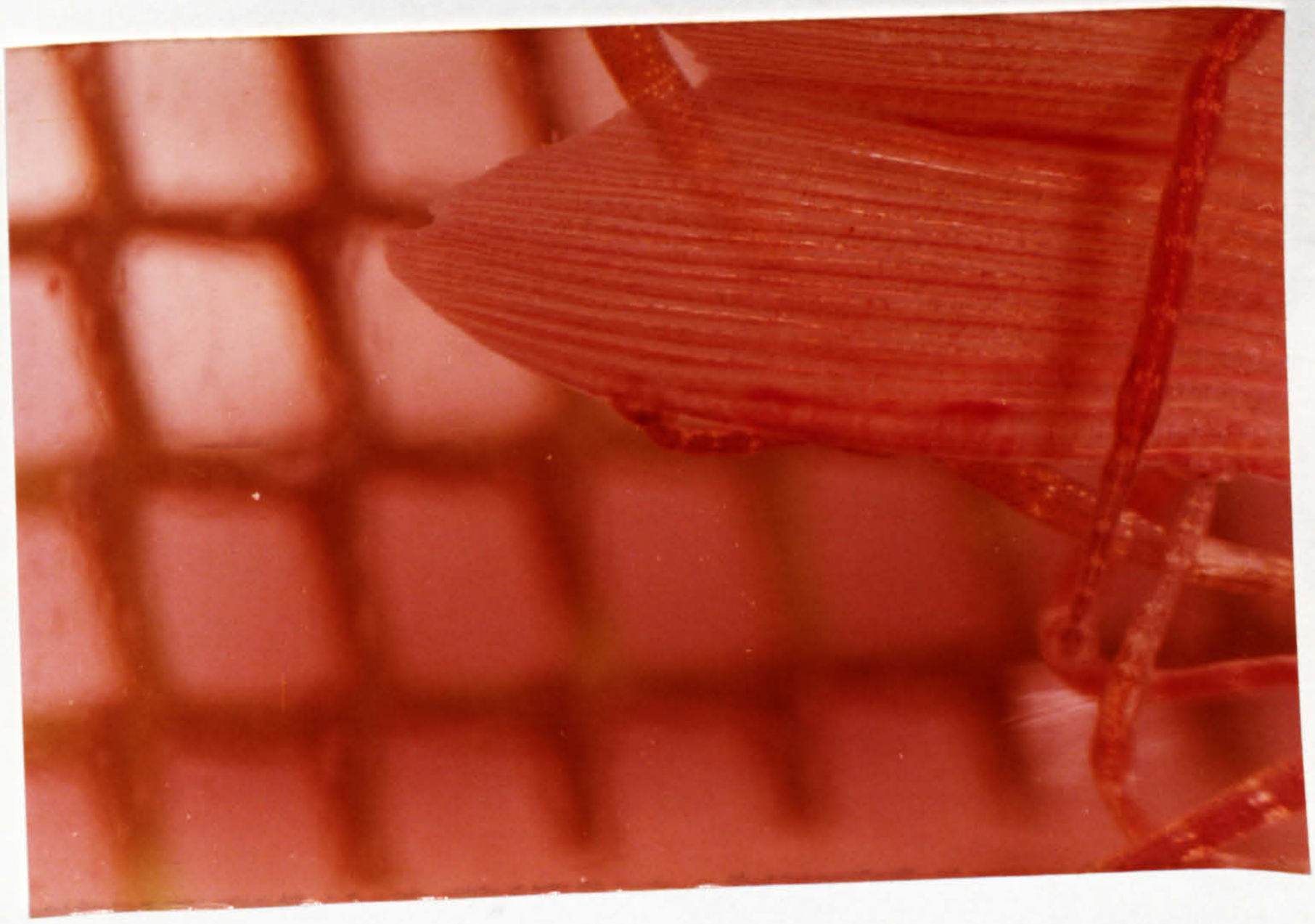
Plate 14

Posterior body and caudal fin of C.carassius to show positions of posterior and anterior suckers of P.geometra.

Plate 15

Close up of caudal fin of C.carassius to show position of the anterior sucker of P.geometra during feeding.





APPENDICES

Appendix 1

Min.Int.	No.	% Parasit.	Emp.P	x	Y	P	r	nP	$(r-nP)^2$	nP(1-P)	$\frac{(r-nP)^2}{nP(1-P)}$	w	nw	nwx	nwx^2	Y	nwy	nwy^2	nwxy
0 - 10	2	13.34	3.88	1.0000	3.75	10.6	2	1.59	0.17	1.42	0.12	0.336	5.04	5.04	5.04	3.89	19.61	76.27	19.61
10 - 20	1	20.01	4.16	1.3010	4.50	31.0	3	4.65	2.72	3.21	0.85	0.581	8.72	11.34	14.76	4.19	36.54	153.09	47.51
20 - 30	4	46.69	4.91	1.4771	4.94	47.6	7	7.14	0.02	3.74	0.01	0.637	9.56	14.12	20.86	4.92	47.04	231.41	69.47
30 - 40	3	66.70	5.43	1.6021	5.26	60.3	10	9.05	0.90	3.59	0.25	0.616	9.24	14.80	23.72	5.43	50.17	272.44	80.36
40 - 50	1	73.37	5.62	1.6990	5.50	69.0	11	10.35	0.42	3.21	0.13	0.581	8.72	14.82	25.17	5.61	48.92	272.44	83.14
50 - 60	1	80.04	5.86	1.7782	5.70	75.7	12	11.36	0.41	2.75	0.15	0.532	7.98	14.19	25.23	5.83	46.52	271.23	82.73
60 - 70	0	80.04	5.86	1.8451	5.86	80.5	12	12.08	0.01	2.36	0.004	0.471	7.07	13.04	24.07	5.84	41.29	241.13	76.15
70 - 80	0	80.04	5.86	1.9031	6.01	84.4	12	12.66	0.44	1.97	0.22	0.439	6.59	12.54	23.87	5.83	38.42	223.99	73.11
80 - 90	0	80.04	5.86	1.9542	6.14	87.2	12	13.08	1.17	1.67	0.70	0.405	6.08	11.88	23.22	5.80	35.26	204.53	68.90
90 - 100	1	86.71	6.11	2.0000	6.25	89.4	13	13.41	0.17	1.42	0.12	0.370	5.55	11.10	22.20	6.12	33.97	207.87	67.93

Snw = 74.52 Snwx = 122.87 $(Snwx^2)$ = 208.14 Snwy = 397.74 $(Snwy^2)$ = 2156.40 Snwxy = 668.91

x = 1.65 y = 5.34 $\frac{1}{snw}$ = 0.01342 $\frac{(Snwx)^2}{Snw}$ = 202.59 $\frac{Snwy \times Snwx}{Snw}$ = 655.80 $\frac{(Snwy)^2}{snw}$ = 2122.88

Sxx = 5.55 Sxy = 13.11 Syy = 33.52

χ^2 = 2.55

b = 2.36 ± 0.42 Y = 1.45 + 2.36x Log ED 50 = 1.51 ED 50 = 32.359 minutes

Appendix 2

Female A.foliaceus

Mo=	5		10		20		50	
t	Pt	∝	Pt	∝	Pt	∝	Pt	∝
0.50	0.80	0.3487	1.40	0.3016	1.60	0.1668	4.80	0.2019
1.00	1.60	0.3857	1.80	0.1985	3.60	0.1985	7.40	0.1602
1.50	1.80	0.2975	2.80	0.2190	4.60	0.1742	10.20	0.1521
2.00	1.80	0.2231	3.00	0.1783	5.00	0.1438	11.60	0.1320
2.50	2.00	0.2043	3.40	0.1682	5.40	0.1259	12.60	0.1161
3.00	2.20	0.1953	3.80	0.1593	6.40	0.1286	13.60	0.1058
3.50	2.40	0.1868	4.00	0.1460	6.80	0.1187	14.20	0.0955
4.00	2.60	0.1835	4.20	0.1362	7.40	0.1155	14.20	0.0835
4.50	2.80	0.1824	4.40	0.1288	8.20	0.1173	14.20	0.0742
5.00	2.80	0.1642	4.60	0.1232	8.40	0.1089	14.40	0.0679
5.50	2.80	0.1493	4.80	0.1189	9.20	0.1120	14.60	0.0628
6.00	2.80	0.1368	5.00	0.1155	9.60	0.1090	14.60	0.0576
6.50	2.80	0.1263	5.20	0.1129	10.00	0.1066	14.60	0.0531
7.00	2.80	0.1173	5.20	0.1049	10.40	0.1049	14.80	0.0501
7.50	2.80	0.1095	5.20	0.0979	10.40	0.0979	15.00	0.0476
8.00	2.80	0.1026	5.20	0.0917	10.40	0.0917	15.00	0.0446
8.50	2.80	0.0966	5.20	0.0863	10.40	0.0863	15.00	0.0420
9.00	2.80	0.0912	5.20	0.0816	10.40	0.0816	15.00	0.0396
9.50	2.80	0.0864	5.20	0.0773	10.40	0.0773	15.00	0.0375
10.00	2.80	0.0821	5.20	0.0734	10.40	0.0734	15.00	0.0357
10.50	2.80	0.0782	5.20	0.0699	10.40	0.0699	15.00	0.0340
11.00	2.80	0.0746	5.20	0.0667	10.40	0.0667	15.00	0.0324
11.50	2.80	0.0714	5.20	0.0638	10.40	0.0638	15.00	0.0310
12.00	2.80	0.0684	5.20	0.0612	10.40	0.0612	15.00	0.0297

Male A.foliaceus

Mo=	5		10		20	
t	Pt	∝	Pt	∝	Pt	∝
0.50	0.80	0.3487	1.00	0.2107	1.00	0.1026
1.00	1.20	0.2744	2.00	0.2231	2.00	0.1054
1.50	1.60	0.2571	2.40	0.1830	3.00	0.1083
2.00	1.80	0.2231	2.60	0.1506	3.60	0.0992
2.50	1.80	0.1785	3.00	0.1427	4.00	0.0794
3.00	2.00	0.1703	3.60	0.1488	4.80	0.0915
3.50	2.20	0.1657	3.80	0.1366	5.00	0.0822
4.00	2.40	0.1635	4.00	0.1277	5.60	0.0821
4.50	2.60	0.1631	4.00	0.1135	5.80	0.0761
5.00	2.80	0.1642	4.20	0.1089	5.80	0.0685
5.50	2.80	0.1493	4.60	0.1054	6.20	0.0675
6.00	2.80	0.1368	4.80	0.1027	7.00	0.0718
6.50	2.80	0.1263	5.00	0.1006	7.00	0.0663
7.00	2.80	0.1173	5.00	0.0990	7.20	0.0638
7.50	2.80	0.1095	5.00	0.0924	7.20	0.0595
8.00	2.80	0.1026	5.00	0.0866	7.20	0.0558
8.50	2.80	0.0966	5.00	0.0815	7.20	0.0525
9.00	2.80	0.0912	5.00	0.0770	7.20	0.0495
9.50	2.80	0.0864	5.00	0.0730	7.20	0.0470
10.00	2.80	0.0821	5.00	0.0693	7.20	0.0446
10.50	2.80	0.0782	5.00	0.0660	7.20	0.0425
11.00	2.80	0.0746	5.00	0.0630	7.20	0.0406
11.50	2.80	0.0714	5.00	0.0603	7.20	0.0388
12.00	2.80	0.0684	5.00	0.0578	7.20	0.0372

20 male A.foliaceus onto different host species

	G.aculeatus		N.barbatulus		C.carassius		P.phoxinus	
t	Pt	∝	Pt	∝	Pt	∝	Pt	∝
0.50	1.00	0.1026	0.20	0.0201	9.00	1.1957	1.80	0.1886
1.00	2.00	0.1054	1.60	0.0834	14.80	1.3471	4.60	0.2614
1.50	3.00	0.1083	1.80	0.0629	16.60	1.1813	5.80	0.2283
2.00	3.60	0.0992	1.80	0.0472	18.20	1.2040	6.60	0.2002
2.50	4.00	0.0794	2.00	0.0421	18.40	1.0103	8.40	0.2179
3.00	4.80	0.0915	2.40	0.0426	18.40	0.8419	10.00	0.2310
3.50	5.00	0.0822	2.60	0.0398	18.40	0.7216	10.40	0.2097
4.00	5.60	0.0821	2.80	0.0377	18.40	0.6314	11.00	0.1996
4.50	5.80	0.0761	2.80	0.0335	18.40	0.5613	11.60	0.1928
5.00	5.80	0.0685	2.80	0.0302	18.40	0.5051	12.20	0.1883
5.50	6.20	0.0675	2.80	0.0274	18.40	0.4592	12.40	0.1759
6.00	7.00	0.0718	2.80	0.0251	18.40	0.4210	12.60	0.1657
6.50	7.00	0.0663	2.80	0.0232	18.40	0.3886	12.60	0.1530
7.00	7.20	0.0638	2.80	0.0215	18.40	0.3608	12.60	0.1420
7.50	7.20	0.0595	2.80	0.0201	18.40	0.3368	12.60	0.1326
8.00	7.20	0.0558	2.80	0.0189	18.40	0.3157	12.60	0.1243
8.50	7.20	0.0525	2.80	0.0177	18.40	0.2971	12.60	0.1170
9.00	7.20	0.0495	2.80	0.0168	18.40	0.2806	12.60	0.1105
9.50	7.20	0.0470	2.80	0.0159	18.40	0.2659	12.60	0.1047
10.00	7.20	0.0446	2.80	0.0151	18.40	0.2526	12.60	0.0994
10.50	7.20	0.0425	2.80	0.0144	18.40	0.2405	12.60	0.0947
11.00	7.20	0.0406	2.80	0.0137	18.40	0.2296	12.60	0.0904
11.50	7.20	0.0388	2.80	0.0131	18.40	0.2196	12.60	0.0865
12.00	7.20	0.0372	2.80	0.0126	18.40	0.2105	12.60	0.0829

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